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College of Applied Medical Sciences

Department of Clinical Laboratories

Study of Some Immunological and Biochemical Markers in Patients with Ischemic Heart Diseases in Kerbala Province

A Thesis

Submitted to the Council of the College of Applied Medical Science - University of Kerbala In Partial Fulfillment of the Requirements for the Degree of Master in Clinical Laboratories

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بِسْمِ اللَّـهِ الرَّحْمَـٰنِ الرَّحِيمِ

﴿ اللَّهُ نُورُ السَّمَاوَاتِ وَالْأَرْضِ مَثَلُ نُورِهِ كَمِشْكَاةٍ فِيهَا مِصْبَاحٌ الْمِصْبَاحُ فِي زُجَاجَةٍ الزُّجَاجَةُ كَأَنَّهَا كَوْكَبٌ دُرِّيٌّ يُوقَدُ مِنْ شَجَرَةٍ مُبَارَكَةٍ زَيْتُونَةٍ لَا شَرْقِيَّةٍ وَلَا غَرْبِيَّةٍ يَكَادُ زَيْتُهَا يُضِيءُ وَلَوْ لَمْ تَمْسَسْهُ نَارٌ نُورٌ عَلَى نُورٍ يَهْدِي اللَّهُ لِنُورِهِ مَنْ يَشَاءُ وَيَضْرِبُ اللَّهُ الْأَمْثَالَ لِلنَّاسِ وَاللَّهُ بِكُلِّ شَيْءٍ عَلِيمٌ ﴾.

صدق الله العلي العظيم

سورة النور، الآية ٣٥

Supervisor Certification

We certify that this thesis which is entitled **(Study of Some Immunological and Biochemical Markers in Patients with Ischemic Heart Diseases in Kerbala Province)** by **(Dalia Jassim Mohammed)** in the Department of Clinical Laboratories \College of Applied Medical science\University of Kerbala in a partial requirement for the Degree of Master of clinical laboratories.

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Dedication

I’d like to dedicate my humble work to whom :

who make me love to learn and lighten my ways and do his best to keep me forward, stand steady, stay strong, my power and courage source and my pride my dear father .

Who taught me to chase my dreams, be ambitious, dear to challenge ،never give up and give the best I can, my Source of tenderness and love, my mother.

who supported me and shared my joys and sorrows, my brothers and sisters.

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Thanks for all

Dalia Jassim

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List of Abbreviations

Abbreviations	Description
4-AP	4-aminophenazone
ACS	Acute coronary syndrome
ADP	Adenosine-5-diphosphate
AMI	Acute Myocardial Infraction
APC	Antigen-presenting cells
APC	Antigen-presenting cells
ASCVD	Atherosclerosis cardiovascular disease
ATP	Adenosine Triphosphate
AVP	Arginine Vasopressin
BMI	Body Mass Index
Bp	Blood pressure
CAD	Coronary artery disease
CCTA	Coronary computed tomography angiography
CHD	Coronary heart disease
CMR	Cardiovascular magnetic resonance
CRP	C-reactive protein
cTnI	Cardiac troponin I

cTn-I	Cardiac troponin I
cTnT	Cardiac troponin T
CVD	Cardiovascular disease
DAP	Dihydroxyacetone phosphate
DM	Diabetes mellitus
ECG	Electrocardiogram
Echo	Exercise stress echocardiography
ELISA	Enzyme-linked immunosorbent assay
ESC	European Society of Cardiology
FFR	Fractional flow reserve
FOXP3	Forkhead Box Protein P3
G3P	Glycerin-3-phosphate
GPO	Glycerol Phosphate Dehydrogenase
HbA1c	Hemoglobin A1c
HDL	High-Density Lipoprotein
HF	Heart failure
HRP	Horseradish Peroxidase
hs-CRP	high-sensitivity C-reactive protein
IHD	Ischemic heart disease
IL-17	Interlukin-17
IL-35	Interlukin-35
IPEX	Immune Dysregulation, Polyendocrinopathy, Enteropathy, X-linked Syndrome
IRA	Infarct related artery
LDL	Low Density Lipoprotein
LV	Left Ventricle
LVEF	Left Ventricle Ejection Fraction

MACEs	Major Adverse Cardiovasculities
MI	Myocardial Infraction
NSTEMI	Non-ST elevation myocardial infarction
OD	optical density
PET	Positron emission tomography
SA	Stable Angina
SD	standard deviation
SIHD	Stable ischemic heart disease
SPECT	the Single Photon Emission Computed Tomography
SPSS	Statistical Package for the Social Sciences
STAT3	Signal Transducer and Activator of Transcription 3
STEMI	ST-elevation myocardial infarction
TAG	Triacylglycerol's
TC	Total Cholesterol
TLR	Target Lesion Revascularization
TMB	Tetramethylbenzidine
TNNI3	Troponin I Type 3
Tregs	Regulatory T-Cells
UA	Unstable Angina
VLDL	Determination of Serum Very Low Density Lipoprotein
VWF	Von Willbrand Factor
WHO	World Health Organization

Summary

Ischemic heart disease((IHD) is a disease of the heart triggered by decreased Oxygen allocation to the myocardium It is mainly instigated due to blockage of arteries by the accumulation of cholesterol on walls. Ischemia is the phrase used to define “reduced blood supply. Coronary arteries supply blood to heart muscle, blockage of coronary artery may start to decrease in the blood supply to heart.

The cross sectional study consist (100) samples divided into two main groups (patients groups (85) and control groups (15) In both sex males and females with age range (25-80 years) Blood sample were collected in a period from March to August 2024 in Imam Al-Hassan Al-Mujtaba Teaching Hospital, and Karbala Center for Heart Diseases and Surgery in Imam Al-Hussein Teaching Hospital in Karbala Province .

In this study, some immunological (Interlukin-35 (IL-35), Interlukin-17A (IL-17A), Forhkead Box P-3 (FOXP3) , and of high-sensitivity C-reactive protein (hs-CRP)), and biochemical (copeptin ,Von Willbrand Factors (VWF),and Troponin I) parameters were monitored in patients and these parameters were determined using enzyme-linked immunosorbent assay (ELISA).

The concentration of troponin I increased significantly ($P<0.00001$) in Myocardial infraction (0.066 ng/ml) compared with Stable angina ,unstable angina (0.041, 0.039 ng/ml) respectively and no significant increase between stable and un stable angina.

The concentration of copeptin gradually increased significant (P < 0.0 0001) in Myocardial infraction (907.75pg/mL) compared with other parameters control ,with risk factors, stable and un stable angina (253.51 ,344.57 ,425.18 , 517.57pg/ml) respectively.

and also unstable angina (517.57 pg/ml) was increased significant (P< 0.05) compared with stable angina (425.18 pg/ml).

The concentration of VWF increased significantly (P<0.00001) in Myocardial infraction (4.57 ng/ml) compared with other parameters (control, with risk factors, Stable angina ,unstable angina (0.24, 0.94, 1.44 , 2.92 ng/ml) respectively and also unstable angina (2.92 ng/ml) is significant increasing (P<0.00001) compared with stable angina (1.44 ng/ml).

The concentration of hs-CRP increased significantly (P<0.00001) in unstable angina and continually highly increasing in myocardial infraction (417.75, 782.60 pg/ml) respectively compared with other parameters (control, with risk factors, stable angina (117.21, 249.77, 200.00 pg/ml) respectively this result indicate hs-CRP as predictor to myocardial infraction .

The concentration of Foxp3 increased significantly (p<0.00001) in unstable angina and continually highly significant increasing in myocardial infraction (5.51 ,10.64 ng/ml) respectively compared with other parameters (control, with risk factors, stable angina) (1.48, 2.83, 3.68 ng/ml) respectively this result indicate the Foxp3 as predictor to myocardial infraction.

The concentration of IL-17A highly increased significantly (p < 0.00001) in unstable angina and continually significant increasing in myocardial infraction (491.64, 944.38pg/ml) respectively compared with

other parameters (control ,with risk factors, Stable angina (91.40, 333.70, 258.56 pg/ml) respectively, this result indicated the IL17 as indicator to myocardial infraction.

The concentration of IL-35 highly decreased significantly ($p < 0.00001$) in myocardial infraction (146.15 pg/ml) compared with group control (405.90pg/ml) and all so decreased significant with other parameters, (with risk factor ,Stable angina ,unstable angina (157.87, 160.96, 160.35pg/ml) respectively.

The study was indicated that increased serum concentrations of hs-CRP, IL-17A, and FoxP3, along with a reduction in IL-35 levels, in patients with stable angina, unstable angina, and myocardial infarction. Furthermore, the concentration of Troponin I was significantly higher in myocardial infarction compared to other conditions (including those control, with risk factors, stable angina, and unstable angina), highlighting its potential as a key biomarker for myocardial injury. Additionally, Copeptin was significantly elevated in unstable angina, and its levels continued to rise significantly in myocardial infarction. The study also suggested that von willbrand factor may serve as a predictive biomarker, as it significantly increased in unstable angina and continued to rise markedly in myocardial infarction.

Chapter one
Introduction

Introduction

Ischemic heart disease IHD is a rapidly increasing common cause of death in the world. Ischemic heart diseases are the leading cause of death in both developed and developing countries , IHD is the most prevalent manifestation and is associated with high mortality and morbidity (Ahmed *et al*.,2022). Ischemic heart disease is a disease of the heart triggered by decreased Oxygen allocation to the myocardium (muscle of the heart). It is mainly instigated due to blockage of arteries by the accumulation of cholesterol on walls. Ischemia is the phrase used to define "reduced blood supply. Coronary arteries supply blood to heart muscle, blockage of coronary artery may start to decrease in the blood supply to heart. Whether or not there will be primarily swift brutal narrowing or closure of either the large coronary arteries or coronary artery finish branches by debris showing downstream in the blood flow(Anusha *et al*.,2020). Plaque can also cause blood clots and blocked blood flow, which can trigger a heart attack then causing death (Suryati and Suyitno.2020). Many risk factor that have greatly increased the incidence of coronary heart diseses , such as hypertension, hyperlipidemia, diabetes, obesity, and aging(shu *et al*.,2024).

Regulatory T cells (T-reg) are components of the adaptive immune system, which differ from naïve T cells upon antigen or cytokine stimulation and principal inflammatory responses that are currently recognized(Dikiy and Rudensky.2023).T-reg express of forkhead box protein P3 (Foxp30). The FOXP3 is a transcription factor responsible for both the differentiation and functioning of T-reg (Ohkura and Sakaguchi.2020) .Malfunctioning of transcription factor Foxp3 caused by

the mutagenesis process affects the development of disorders of the immune response and autoimmune diseases (Mertowska *et al.*,2022).The expression of FOXP3 isoforms affects T-reg cell function. Reduced Treg cell function has been associated with coronary artery disease (CAD) (Lundberg *et al*.,2017).

Interleukin 17 (IL-17) is an important cytokine that has several roles in host defense responses against mucosal infections; additionally, it is a primary cytokine and therapeutic target in a number of autoimmune, inflammatory illnesses, and cancers. The functions of IL-17 in vivo are not only limited to inflammation, but are also closely associated with both physiological and pathological processes(Huangfu *et al*.,2023).IL-17 promotes recruitment of inflammatory cells to the luminal wall, leading to the development of plaque and cardiovascular events, elevated levels are associated with ACS (Liuzzo *et al*.,2013).

interleukin-35 (IL-35) is a recently identified cytokine in the IL-12 family, which plays a key role in the suppressive function of regulatory T cells (Tregs) (Ye *et al*.,2021). Interleukin (IL)-35 regulates T and B cell-mediated pro-inflammatory and anti-inflammatory immune responses. IL-35 suppresses conventional T cells and promotes their conversion to IL-35-inducible regulatory T cells (iTr35 cells), which play a vital role in immune regulation as they inhibit various immune responses due to the suppressive effects of IL-35. It can contribute to the induction of iTr35 cells and regulatory B (Breg) cell populations and induces anti-inflammatory effects via the inhibition of type 1 helper T (Th1) cell, Th2 cell and Th17 cell responses(Zhu and Shan.2020). IL-35 appears to play an important regulatory role in a variety of cardiovascular diseases,

autoimmune diseases, tumours, and inflammatory diseases(Zhang and Xing .2023).plasma IL-35 levels in patients with acute coronary syndrome and stable angina were compared to levels in controls with chest pain. They found a positive correlation between lower plasma IL-35 levels and left ventricular ejection fraction(Neubauer *et al* .,2020).

Von Willebrand factor (VWF) is a large multimeric plasma protein that plays a major role in hemostasis . First, VWF recruits platelets to sites of vascular injury by forming a bridge between the damaged vessel wall and platelets. Second, VWF also serves as a carrier protein for coagulation factor VIII (FVIII) and hence protects FVIII from degradation, cellular uptake or binding to the surface of activated platelets and endothelial cells .VWF is produced exclusively by endothelial cells and megakaryocytes (Denorme *et al* .,2019).VWF it is have role in coagulation , and plasma levels of VWF are increased with age. Elevated VWF promotes thrombosis, atherosclerotic plaque formation, inflammation and proliferation of vascular smooth muscle cells(Wang *et al*.,2023a).Impairment of the hemostatic role of VWF may contribute to the development of CAD and its complications. In addition, VWF may contribute to inflammation in atherosclerosis. VWF facilitates leukocyte recruitment and extravasation at high shear rates . VWF may modulate the inflammatory response at the sites of atherosclerotic lesions through this function(Kozlov *et al*.,2022).Elevated VWF levels have been associated with an increased risk of ischemic heart disease (IHD) (Mihyawi *et al*.,2022).

Troponins are proteins that regulate muscle contraction . In the myocardium the subunits are cardiac troponin I (cTnI), cardiac troponin T

(cTnT) and cardiac troponin C. All three are integral components of the contractile mechanism of cardiac muscle . They have separate genes, which differentiate them from skeletal muscle troponin . Immunoassays have been developed for both cTnI and cTnT and either of these troponins can be used in the investigation of possible myocardial injury or infarction. cardiac troponins are specific for myocardial tissue (Potter *et al*.,2022). Troponin T concentrations are associated with worse diastolic function and that associated impairments in diastolic function partially account for the heightened risk of heart failure (Myhre *et al*.,2019).

Copeptin is a 39-amino acid glycosylated peptide (Jalleh and Torpy .2021), it is the carboxylterminus of the arginine vasopressin (AVP) precursor peptide. The main physiological functions of AVP are fluid and osmotic balance, cardiovascular homeostasis, and regulation of endocrine stress response. Copeptin, which is released in an equimolar mode with AVP from the neurohypophysis, has emerged as a stable and simple-to-measure surrogate marker of AVP and has displayed enormous potential in clinical practice . Copeptin is a diagnostic and prognostic biomarker in CVD, including the rapid rule-out of acute myocardial infarction (AMI), mortality prediction in heart failure (HF), and stroke (Mu *et al*.,2022). Copeptin has also been investigated for its role in prognosticating heart failure. Copeptin is a strong biomarker for mortality and morbidity in patients with heart failure after acute myocardial infarction (Jalleh and Torpy.2021). Elevated copeptin predicts development of HF in older adults (Schill *et al*.,2021).

Aim of The Study:

This study aim to evaluate and compare some immunological and biochemical markers in patients with and without ischemic heart diseases in Karbala provinces:

1. The level of immunological markers Interleukin 35, Interleukin 17A, FOXP3,and hs-CRP investigated in Individuals with and without ischemic heart disease.
2. The level of new biochemical markers, copeptin and von Willebrand factor (VWF), and their relationship with and without ischemic heart disease were determined.
3. The level of routine biochemical markers, Troponin I, a lipid profile, and HBA1c, and their relationship with and without ischemic heart disease, were determined.

Chapter Two
Literature Review

2.1 Ischemic Heart Disease

Ischemic heart disease IHD is a rapidly increasing common cause of death in the world. This disease is the insufficient status of oxygen within the cardiac muscles due to an imbalance between oxygen supply and demand, and a cardiac disease that occurs as a result of coronary artery stenosis. Ischemic heart disease diseases are the leading cause of death in both developed and developing countries ,among these IHD is the most prevalent manifestation and is associated with high mortality and morbidity (Ahmed *et al* .,2022). The ischemic heart disease is a disease of coronary arteries, so it is also called coronary heart disease (CHD) or coronary artery disease (CAD) or in which there is a blood supply reduction to the myocardium, mostly due to atherosclerosis in the coronary arteries (Aadai *et al*., 2021). CAD is a common heart condition in which we can observe the narrowing or blockage of major blood vessels coronary arteries. CAD is caused primarily by plaque formation within the intima of the vessel wall, with plaque being defined as a fatty material growing inside intima along with a severe inflammation, especially if the inflammation is chronic .This in turn causes difficulties in supplying the cardiomyocytes with enough blood ،oxygen, and nutrients (Shao *et al*., 2020).

According to the European Society of Cardiology (ESC) CAD is defined as an episode of a reversible incommensurability between the nutrient needs of the cardiac muscle and its demand that is associated with ischemia or hypoxia (Kasprzyk *et al*., 2018). Risk factors increase of affected ischemic heart disease older age, gender , diabetes mellitus , hypertension , and hyperlipidemia (Gheisari *et al*., 2020) .

Cardiovascular disease (CVD) encompass a wide spectrum of disorders, including diseases of the heart muscle and the vascular system that supplies the brain, heart, and other vital organs with blood and oxygen. CVDs are the preeminent cause of death worldwide (Kaufman *et al* .,2023). CVD is an umbrella term for a number of inter-linked diseases, generally defined as coronary artery disease, cerebrovascular disease, high blood pressure, peripheral arterial disease, rheumatic and congenital heart diseases, arrhythmia. This burden of CVDs can be decreased by careful risk reduction (such as lifestyle modification, smoking and alcohol cessation, weight optimization, physical exercise), and proper medical treatments, including herbal components. The prevention of CVDs can reduce the occurrence of major cardiovascular events, thereby reducing premature disability, morbidity, and mortality, while prolonging survival and quality of life (Shah *et al* ., 2024).

2.2 Epidemiology

Ischemic Heart Diseases is the number one cause of death, disability, and human suffering globally. Worldwide, 126 million people suffer from IHD, with 9 million deaths resulting from this condition per year.Eastern European countries are sustaining the highest prevalence.

Coronary heart diseases is the leading cause of morbidity, mortality, and disability in the Middle East and North Africa (Manla and Almahmeed .,2023).Among the 21 countries in the Middle East, in countries including Iran and Saudi Arabia with the highest prevalence and increasing prevalence of IHD (Ahmadi *et al*.,2023).

In Iraq, the epidemiological data on the incidence and prevalence of CAD as evidence of awareness are limited due to the unavailability of evidence-based national guidelines for the management of cardiovascular

disease and surveillance studies as compared to other Eastern Mediterranean countries(Amen *et al* ., 2020).

According to the latest WHO data published in 2020, Coronary Heart Disease death in Iraq reached 36,594 or 24.98% of total deaths (World health rankings .2021).

2.3 Etiology

Coronary artery disease is a multifactorial phenomenon. Etiologic factors can be broadly categorized into non-modifiable and modifiable factors. (Shahjehan and Bhutta,2024). Non-modifiable risk factors are Age, Gender and Family history. Modifiable risk factors have a smaller but still significant role these include hypertension, hyperlipidemia, diabetes, obesity and smoking (Brown *et al*., 2020).

2.3.1 Non-modifiable risk factors

2.3.1.1 Age: Age is the strongest factor related to the development of coronary heart disease(Madhavan *et al*.,2018). Age is also the strongest predictor of IHD in patients aged ≥ 65 years who have 15 times the odds of IHD compared to patients younger than 45 years . Ageing also correlates with the acquisition and increments in other major modifiable risk factors to contribute to the development of IHD (Albakri .2018).

2.3.1.2 Gender : men have conventionally experienced an approximate 2-fold greater incidence of CHD and related mortality than women, but the gap in morbidity narrows with increasing age as elderly women experience greater incidences of heart disease (Taylor *et al*.,2018). Another possible risk factor in aging individuals is that older females have a higher risk of cardiovascular disease compared to men of the same age .Nevertheless, the risks of cardiovascular disease (CVD) rise in both

men and women .As individuals age, there is a general decrease in sex hormones, including estrogen and testosterone. However, hormone replacement therapy are generally shown to be ineffective in improving outcomes in elderly individuals and may potentially elevate the likelihood of cardiac events in older persons (Rodgers *et al*., 2019).

2.3.1.3 Family History : Family history of CVD is an independent risk factor for premature CHD. The risk of premature CHD increases linearly with increase in number of affected family members. Collecting family history beyond parental history of CVD is important for risk stratification. Targeting young individuals with family history of CVD for intensive risk reduction interventions may help to prevent future events(Chacko *et al*., 2020).

2.3.2 Modifiable Risk Factors

2.3.2.1 Smoking: smoking affects of public health. that there are adverse impacts on several physiological systems, particularly on general vessels, large vessels, and coronary vessels.. An connection was discovered between excessive smoking and acute coronary syndrome(Thygesen *et al*., 2019).

2.3.2.2 Dyslipidemia: dyslipidemia is one of the major risk factors. Hypercholesterolemia is the most common form of dyslipidemia(Du and Qin .2023) The plasma levels of total cholesterol and low-density lipoprotein (LDL) cholesterol are important risk factors for coronary heart disease (Alloubani *et al*.,2021). Dyslipidemia, unhealthy levels of one or more kinds of lipid in blood is a risk factor for IHD. The principal lipoprotein transporting cholesterol (LDL cholesterol) is directly associated with IHD and LDL cholesterol levels in young adulthood predicts development of IHD later in life. LDL is a major contributor to

the pathogenesis of atherosclerosis and LDL cholesterol lowering drugs reduce the risk of IHD by 50% in individuals aged 40 and 30% at the age of 60 years. Highdensity lipoprotein (HDL) cholesterol also correlates closely and inversely with the risk of ischemic heart failure (HF) and is more predictive in men than in women(Hajar, 2017).

unhealthy diet high in saturated fats, cholesterol, salt and sugar may intensify other risk factors such as hypercholesterolemia, obesity and diabetes (Malakar *et al*.,2019).

2.3.2.3 Hypertension: hypertension is known to be a major independent risk factor for CAD. There is a strong progressive association between blood pressure and age-specific mortality from CAD, the a 20 mm Hg increase in systolic BP or 10 mm Hg increase in diastolic BP is associated with roughly twice the risk of death from CAD for patients aged 40 to 69 (Duggan *et al* .,2022).

2.3.2.4 Obesity: Overweight and obesity are conditions where there is either abnormal and/or excessive fat accumulation in the body that predisposes to adverse health especially through its effects on the heart . Obesity was traditionally defined as an increase in body weight greater than 20% of an individual's ideal body weight—the weight associated with the lowest risk of death, as determined by age, height, and sex. Based on these factors, overweight was define(Badreldeen *et al*.,2023). The World Health Organization (WHO) defines overweight as a BMI ≥25 kg/m2 and obesity as a BMI ≥30 kg/m2 (Haam *et al*.,2023). Obesity significantly increases the risk of developing several heart conditions, including coronary artery disease, hypertension, heart failure, stroke, dyslipidemia, diabetes, and atrial fibrillation (Pakhare and Anjankar .2024). Overweight people have been demonstrated to have higher

rates of cardiovascular disease (CVD) mortality and morbidity, particularly when there is central adipose tissue accumulation. It has been demonstrated that abdominal obesity is a global risk factor for CVD. obesity and weight gain are important risk factors for myocardial infarction and ischemic heart disease, which lead to poor outcomes for patients and an increase in the death (Sapra *et al*.,2022).

2.3.2.5 Diabetes Mellitus : is a disease of inadequate control of blood levels of glucose.it is a metabolic disease, involving inappropriately elevated blood glucose levels (Sapra and Bhandari .2023). identify diabetes as a major cardiovascular risk factor(Takamura *et al*.,2022). It increases the risk of clinical atherosclerotic disease by two to three-folds with a higher risk among females. The American Heart Association provides statistics establishing the relationship between diabetes and IHD – at least 68% of diabetic individuals > 65 years die from heart diseases and 16% of stroke and diabetic adults have a two fold increase to die from heart diseases compared to non-diabetic patients (Albakri. 2018). The pathophysiology of myocardial ischemia in diabetic patients is complex and not fully understood. Some diabetic patients have mainly coronary stenosis obstructing blood flow to the myocardium; others present with coronary microvascular disease with an absence of plaques in the epicardial vessels. Ion channels acting in the cross-talk between the myocardial energy state and coronary blood flow may play a role in the pathophysiology of IHD in diabetic patients. In particular, some genetic variants for ATP-dependent potassium channels seem to be involved in the determinism of IHD (Shukhratovna *et al*.,2022).One of the most devastating consequences of DM is its effect on atherosclerosis cardiovascular disease (ASCVD). Approximately two-thirds of those with

DM will die from a myocardial infarction or stroke (Wannamethee *et al*., 2011).

2.4 Classification of Ischemic Heart Diseases

Classification of coronary artery disease is typically done as under (Shahjehan and Bhutta .2023):

1. Stable ischemic heart disease (SIHD)
2. Acute coronary syndrome (ACS) Figure (2.1)

- ST-elevation myocardial infarction (STEMI)
- Non-ST elevation myocardial infarction (NSTEMI)
- Unstable angina

2.4.1 Stable Ischemic Heart Disease (SIHD)

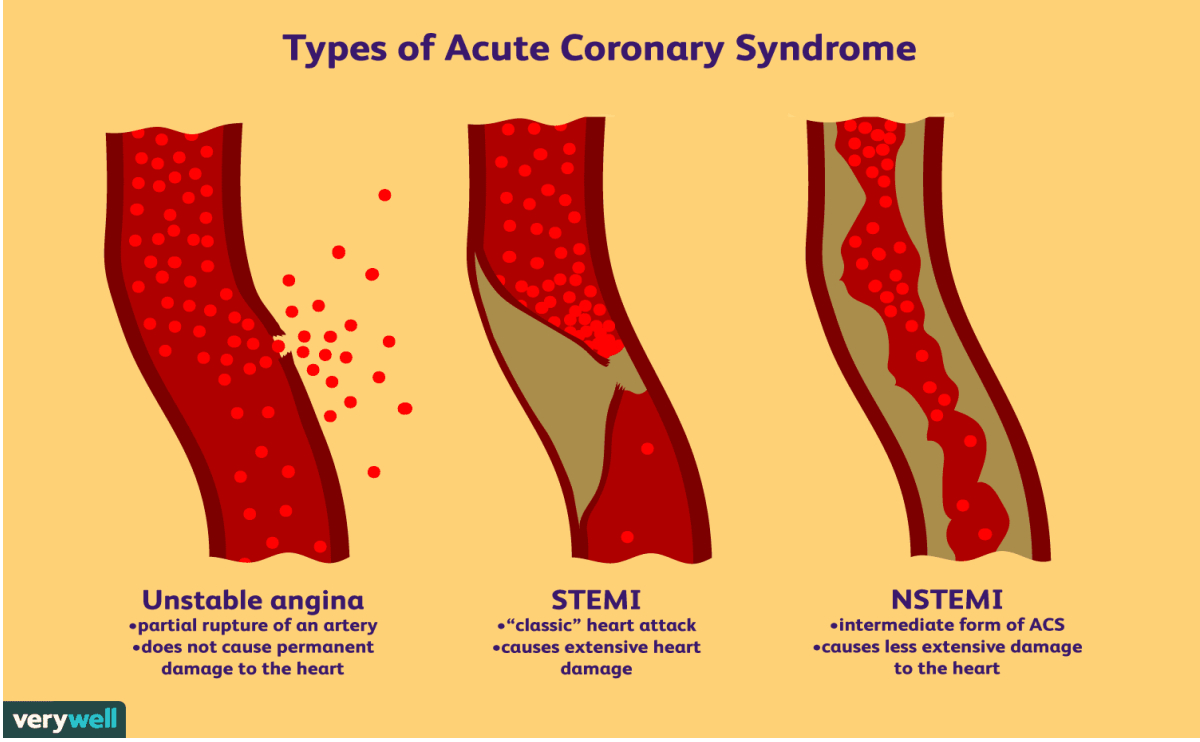
The term stable ischemic heart disease (SIHD) is often used synonymously with chronic coronary artery disease (CAD) and encompasses a variety of conditions where the end result is a repetitive mismatch between myocardial oxygen supply and demand (Dababneh and Goldstein. 2018).This most frequently is seen when long-standing atherosclerotic obstruction within the epicardial coronary arteries results in poor flow and ischemia distally. However, this is not the only mechanism. Various pathophysiologic processes such as coronary artery vasospasm, microcirculation dysfunction, or congenital anomalies can cause the same supply-demand mismatch and result in chronic repetitive ischemic (Gurgoglione *et al*., 2024). IHD remains a major public health problem and is considered as one of the leading causes of mortality worldwide. Unfortunately, the prevalence of CAD is increasing worldwide, especially in the younger population, with increasing

cardiovascular risk factors such as increases in the prevalence of obesity, diabetes mellitus, metabolic syndrome, and physical inactivity. Angina pectoris is the initial manifestation of SIHD and is the most common presentation in these patients (Kyavar and Alemzadeh. 2022). typical angina usually presents as chest discomfort or anginal equivalent that is provoked with exertion and alleviated at rest or with nitroglycerin. Anginal equivalents vary, however, commonly can be described as shortness of breath, nausea, or fatigue that is out of proportion to the activity level. Typical angina is often described as pressure-like, heaviness, tightness, or squeezing. Most commonly, it will affect a broad area of the chest rather than a specific spot. There may be radiation of the pain, depending on which dermatomes are affected.Symptoms will be described as more severe with states of increased demand (i.e., walking, lifting, emotional stress, etc.) Symptoms generally last for two to five minutes, and relief is experienced when the provoking activity is stopped, or the patient takes treatment (Gillen and Goyal *et al*.,2022).

2.4.2 Acute Coronary Syndrome (ACS)

It refers to a group of conditions that include ST-elevation myocardial infarction (STEMI), non-ST elevation myocardial infarction (NSTEMI), and unstable angina. It is a type of coronary heart disease (CHD) (Zègre *et al*.,2019), which is responsible for one-third of total deaths in people older than 35. Some forms of CHD can be asymptomatic, but ACS is always symptomatic (Kerneis *et al*.,2019).The underlying pathophysiology in ACS is decreased blood flow to part of heart musculature which is usually secondary to plaque rupture and formation of thrombus. Sometimes ACS can be secondary to vasospasm with or without underlying atherosclerosis. The result is decreased blood flow to

a part of heart musculature resulting first in ischemia and then infarction of that part of the heart (Singh *et al*.,2022).



Figure(2.1):Types of Acute coronary syndrome (ACS) (Zègre *et al*., 2019).

2.4.2.1 ST-elevation myocardial infarction (STEMI)

ST elevation myocardial infarction (STEMI) is a type of acute coronary syndrome that usually occurs after atherosclerotic plaque rupture or plaque erosion leading to persistent and complete thrombotic occlusion of the infarct-related artery (IRA). A diagnosis of acute STEMI is usually based on symptoms and signs consistent with myocardial ischaemia (i.e. persistent chest pain radiating to the neck, lower jaw or left arm, and ST segment elevation or new left or right bundle branch block on a 12-lead electrocardiogram (ECG). However, some individuals (especially those who are elderly, diabetic and female) present with atypical symptoms such as epigastric pain, shortness of breath, nausea/vomiting, fatigue, palpitations or syncope (McNaughton *et al*.,2022). STEMI is the most

acute manifestation of CAD, with substantial morbidity and mortality (Vogel *et al.*,2019).An acute STEMI is an event in which transmural myocardial ischemia results in myocardial injury or necrosis. The myocardial infarction requires the confirmation of the myocardial ischemic injury with abnormal cardiac biomarkers. It is a clinical syndrome involving myocardial ischemia, EKG changes and chest pain(Akbar *et al*.,2018).

2.4.2.2 Non-ST Elevation Myocardial Infarction (NSTEMI)

Non-ST Elevation Myocardial Infarction and unstable angina are very similar, with NSTEMI having positive cardiac biomarkers. While the cause of this mismatch in STEMI is nearly always coronary plaque rupture resulting in thrombosis formation occluding a coronary artery, there are several potential causes of this mismatch in NSTEMI. There may be a flow-limiting condition such as a stable plaque, vasospasm as in Prinzmental angina, coronary embolism, or coronary arteritis (Suling.2020).Non-coronary injury to the heart such as cardiac contusion, myocarditis, or presence of cardiotoxic substances can also produce NSTEMI. conditions relatively unrelated to the coronary arteries or myocardium itself such as hypotension, hypertension, tachycardia, aortic stenosis, and pulmonary embolism lead to NSTEMI because the increased oxygen demand cannot be met (Basit *et al*.,2023).

2.4.2.3 Unstable Angina

Unstable angina is chest discomfort or pain caused by an insufficient flow of blood and oxygen to the heart. It is part of the acute coronary syndromes and may lead up to a heart attack (Goyal and Zeltser .2022). Diagnosis of UA is based on symptoms suggestive of myocardial ischemia and the absence of acute myocardial injury or necrosis (i.e., no

dynamic elevation of cardiac troponin) . UA should be suspected when the patient presents with resting angina for >20 minute, new onset angina, or crescendo angina, defined as a change from previous episodes in severity, intensity, or duration,which may occur with minimal physical exertion . The ECG may show ST-segment depression, transient (≤20 min) ST-segment elevation, T-wave inversion, or may be normal (Kristensen *et al* .,2022).

2.5 Symptoms of Ischemic Heart Disease

Coronary heart disease is a multifactorial, immunoinflammatory disease of the arteries driven by lipids. Risk factors, such as smoking, hypertension, diabetes mellitus, male gender and inflammation accelerate the process where lipids enter the intima and atherosclerotic plaque develop in the coronary arteries. Reduced blood flow in the coronary arteries due to atherosclerotic luminal narrowing and endothelial dysfunction creates an imbalance between oxygen demand and supply in the myocardium causing(Rebekka *et al* .,2020). Signs and symptoms of ischemic cardiomyopathy incorporate suddenfatigue , shortnessofbreath , dizziness , andpalpitations . The narrowing of coronary arteries reduces the supply of oxygen-rich blood flowing to the heart, which becomes more pronounced during strenuous activities during which the heart beats faster. For some, this causes severe symptoms while others experience no symptoms at all (Polampelli.2020).Complications Arrhythmias, acute coronary syndrome, congestive heart failure, mitral regurgitation, ventricular free wall rupture, pericarditis, aneurysm formation, and mural thrombi are the main complication associated with coronary artery disease (Michniewicz *et al.,* 2018).

2.6 Diagnostics of Ischemic Heart Disease

2.6.1 Biochemical Test of Ischemic Heart Disease

2.6.1.1Troponin

Human cardiac troponin core complex (52kDa core) shown as a ribbon diagram in a calcium-saturated state. Troponin C, troponin I, and troponin T are three regulatory proteins that make up the troponin complex . Crucial for muscle contraction in skeletal and cardiac muscle, but not in smooth muscle (Awuchi *et al*., 2020). Cardiac-specific troponins I and T measurements are commonly utilized as diagnostic and prognostic indications (Mujamammi *et al*.,2020). In figure (2.2) showed the troponin is bound to tropomyosin and it is located in the groove between actin filaments in muscle tissue. Tropomyosin inhibits the attachment point for the myosin cross bridge in a relaxed muscle, hence impeding contraction(Elmisbah and Aiderous, 2018). When a muscle cell is induced to contract by an action potential, calcium channels in the sarcoplasmic membrane open and release calcium into the sarcoplasm. Calcium binds to troponin, leading to a change in its structure form, revealing attachment points for myosin (active sites) on the actin filaments (Cretoiu *et al.,* 2018). Myosin binding to actin initiates cross-bridge creation, leading to muscle contraction. Ca+2 binds to Troponin C, stabilizing the active state and causing Troponin I to detach from actin. Troponin T secures the complex to tropomyosin .Troponin is present in both skeletal and cardiac muscle, however the isoforms of troponin vary across the two muscle types. In skeletal muscle, the Tn C subunit of troponin has four calcium ionbinding sites, while in cardiac muscle there are only three. The precise quantity of calcium that attaches to troponin has not been conclusively determined (Al-khateeb, 2021).

cTnI is exclusively expressed in the myocardium during human development, lacking expression in any skeletal muscle type irrespective of developmental or disease-related stimuli. This exclusive myocardial specificity renders Cardiac troponin I (cTnI) as a marker with high sensitivity and specificity for detecting acute myocardial infarction (AMI) (Sari.2024).Among individuals with suspected acute coronary syndrome, those with a persistently elevated hsTnI concentration consistently had the highest risk of being diagnosed with myocardial infarction and undergoing coronary revascularization. The risk of these outcomes was, for the most part, also positively associated with the magnitude of hsTnI change.Although cTnT and cTnI are expressed in heart tissue in approximately equal amounts in patients with myocardial necrosis, such as in acute myocardial infarction (MI), cTnI frequently reaches peak levels ten times higher than cTnT (Eggers *et al*.,2022).

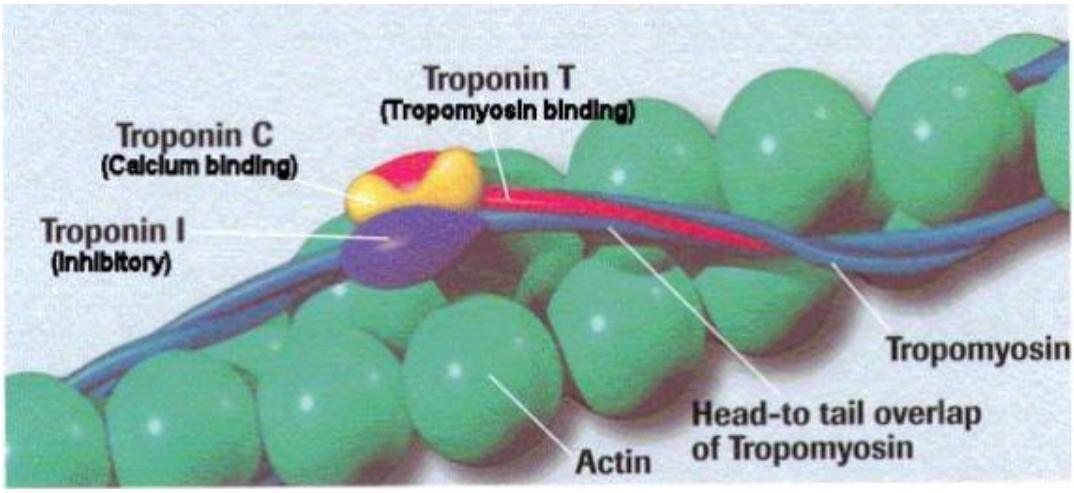


Figure (2.2) : Structure of Troponin (Marston and Zamora, 2020).

Role of Troponin in Ischemic Heart Diseases

Troponins (Tn) are a group of regulatory proteins localized to the myofibrillar apparatus, which regulate excitation-contraction coupling,

and are released into the bloodstream during myocardial injury. Accurate measurement of serum Tn levels plays a central role in the evaluation of patients presenting with chest pain concerning for acute coronary syndrome (ACS) (Maayah *et al*.,2024).Circulating cardiac troponin (cTn) concentrations have become an increasingly important biomarker for the noninvasive detection of myocardial injury in many cardiovascular diseases, in particular, patients with coronary heart disease presenting with acute chest pain (Ni and Wehrens ,. 2018). troponin is better at short-term morbidity and death prediction(Taghdiri. 2024). cardiac troponin I levels were independently associated with the higher severity of CAD. Cardiac troponin I level was also a significant predictor of accidental death, cardiovascular death, MI, a higher level of cardiac troponin I was associated with baseline coronary atherosclerosis, faster progression of CAD, and a higher risk of mortality from all acute cardiovascular events. (Samman *et al* ., 2018).

2.6.1.2 Von Willebrand Factor(VWF)

A multimeric protein that assists in hemostasis by enhancing platelet adhesion and thrombus formation following vascular damage. Von Willebrand disease is characterized by deficiencies in VWF, resulting in a bleeding disorder . VWF is present in the blood, platelets, and endothelial cells. WeibelPalade bodies in endothelial cells store ultra large VWF (UL-VWF) (Rutten *et al.*, 2015).VWF is produced in vascular endothelial cells and then secreted into the bloodstream as abnormally large VWF multimers, which exhibit strong biological activity when interacting with platelets .The VWF multimers are quickly broken down into smaller units by the enzyme ADAMTS13, which is a metalloproteinase that targets and cleaves multimeric VWF between Tyr1605 and Met1606 in the VWF A2 domain. ADAMTS13 loss-offunction mutation causes Upshaw-Schulman

syndrome(Horii *et al.,* 2008). VWF is present in the blood in either a globular or unfolded shape. The structure of VWF is influenced by the shear rate of blood flow in arteries. The rate of shear the rate of change of velocity between fluid layers is measured in inverse seconds (s-1). (Okhota *et al*., 2020). VWF also interacts with blood coagulation factor VIII, serving as its transporter in the bloodstream. Aside from maintaining homeostasis, VWF has been identified as a crucial regulator in angiogenesis, inflammation, and cell processes . The VWF gene is situated on chromosome 12p and consists of 52 exons covering about 178 kb of genomic sequence. Von Willebrand disease, a bleeding ailment that delays the blood clotting process, is caused by mutations in the VWF gene (Li *et al*., 2015).

Von Willebrand Factor in Ischemic Heart Disease

Von Willebrand Factor is released from endothelial cells in response to damage or stress. Elevated levels of VWF are indicative of endothelial dysfunction, a key factor in the development of atherosclerosis (Kozlov *et al*., 2022)..VWF facilitates the adhesion of platelets to the subendothelial matrix at sites of vascular injury. This is particularly important in coronary arteries where high shear rates are present(Lip and Blann.,1997).VWF is crucial for platelet attachment and clumping in areas with fast-flowing blood, such as in narrowed or damaged coronary arteries with plaque buildu In the context of CHD, plaque rupture exposes the subendothelial matrix, leading to platelet adhesion mediated by VWF. This can result in thrombus formation and subsequent myocardial infarction(Spiel *et al* ,.2008). Elevated VWF levels have been associated with an increased risk of CHD events. Studies have shown that individuals with higher VWF levels have a greater likelihood of experiencing adverse cardiac events. (Whincup *et al*.,2002).

2.6.1.3 Copeptin

Arginine Vasopressin (AVP) is one of the key hormones in the human body. AVP is clinically important because it maintains body fluid balance and vascular tone. Unfortunately, AVP laboratory measurements are always difficult and with low accuracy (Abdelmageed and Güzelgül,.2023). Copeptin is a 39 amino acid glycopeptide cleavage product of vasopressin synthesis with high stability (Jalleh and Torpy .2021) show in Figure (2.3). Copeptin and AVP are secreted from the neurohypophysis upon hemodynamic or osmotic stimuli(Christ.2019).The physiological role of copeptin is not fully defined.However, studies have shown that it is a chaperone-like molecule for pro- Arginine Vasopressin (AVP) formation and that it monitors protein folding and interacts with many glycosylated proteins through interaction with the calnexin/calreticulin system, thus increasing the formation of active hormone and decreasing the production of inactive hormone(Christ *et al*, .2022).

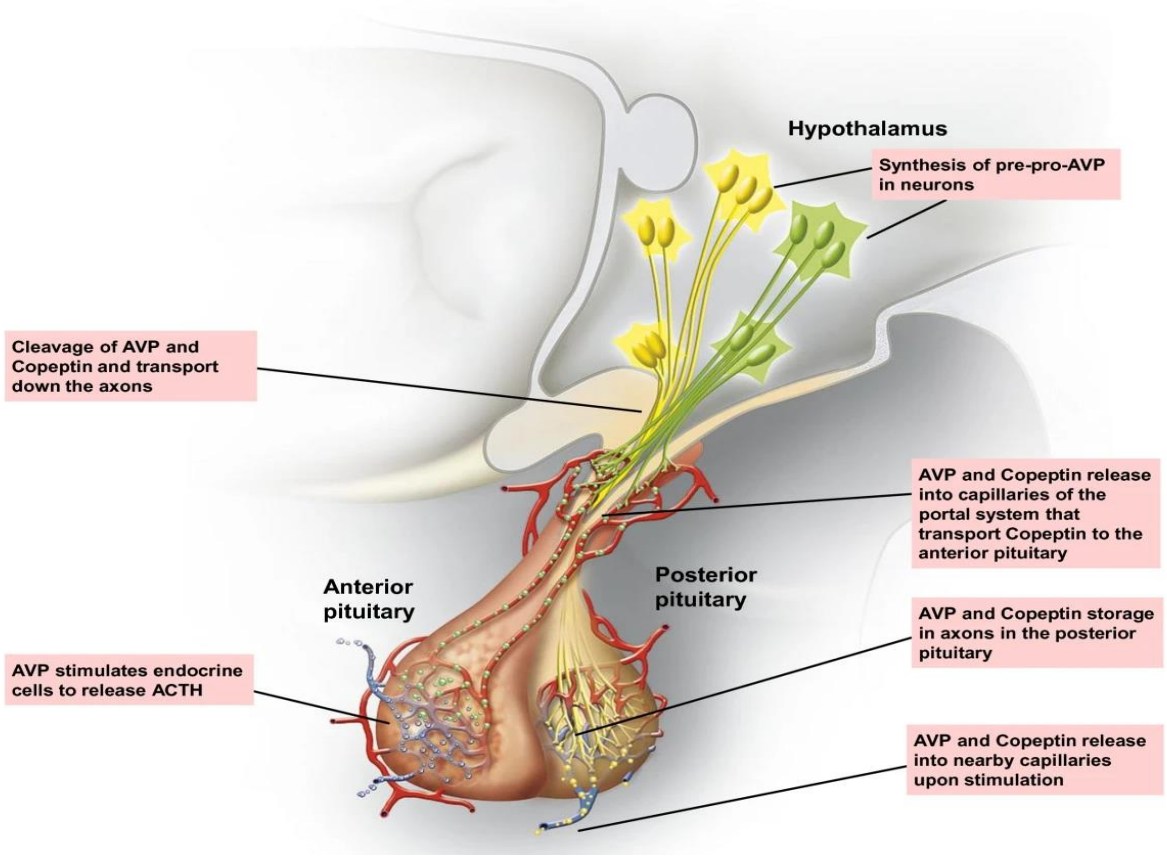


Figure (2.3) :Synthesis of Arginine Vasopressin (AVP) and copeptin in the magnocellular neurons of the hypothalamus and storage in the posterior pituitary(Christ.2019).

AVP plays a pivotal role in the endocrine stress response by stimulating adrenocorticotrophic hormone release and in osmotic and cardiovascular homeostasis by promoting water conservation in the body via the kidney. It is predominantly produced in the hypothalamus but also in other tissues like the sympathetic ganglia, adrenal glands, and testes. The short plasma half-life of AVP of 5-20 min, high instability in plasma even when frozen, and high degree of platelet binding (over 90%) requiring complete pre-analytical removal of platelets make AVP difficult to measure (Refardt *et al*.,2019).Copeptin is cleaved from the same precursor as arginine vasopressin and is released in equimolar amounts with arginine vasopressin from the posterior pituitary in response to the same stimuli.

Its level of stability in the blood, quick and simple analysis, and ease of automation make it much easier to analyze than arginine vasopressin, thereby offering a suitable alternative to measuring arginine vasopressin in endocrine disorders (Moodley .2023).The half-life of copeptin was around 2 times higher than the half-life of AVP, reflecting the differing volume distribution and metabolic clearance rates of the two peptides (Fenske *et al*.,2018).Due to its high ex vivo stability and simple and robust measurement, copeptin offers a simple alternative method to assess the release of AVP indirectly(Refardt *et al*.,2024).

Role of copeptin in Ischemic Heart Diseases

Copeptin is a diagnostic and prognostic biomarker in CVD (Mu *et al*.,2022).It is product of vasopressin synthesis with high stability (Jalleh and Torpy .2021).Copeptin serves as a well-established biomarker for heart diseases and also stands as a predictor of mortality instead of AVP . A recent study has shown that copeptin could predict the development of CAD and CV mortality (Bhatnagar and Jain. 2024) .A high plasma level of copeptin is associated with higher risks of mortality in patients with CAD. Measuring copeptin may be helpful for risk stratification in patients with CAD (Shi and Qian .,2023).

2.6.2 Immunological Test of Ischemic Heart Disease

2.6.2.1 Regulatory T-Cells (T-Reg) FOXP 3

Regulatory T (T-reg) cells are a unique type of cells in the adaptive immune system that are crucial for preventing deadly autoimmune and inflammatory diseases. T-reg cells are the main protectors of the immune system because they can control all known forms of inflammatory responses by influencing various cells in the innate and adaptive immune

system. T-reg cells have many ways of affecting different molecular and cellular targets, giving them a wide range of control over immunity and inflammation. T-reg cells are not only known for suppressing autoimmunity but also play a role in tissue maintenance, repair, and regeneration in both normal and abnormal situations.

Human regulatory T cells are a diverse group heterogeneous defined by the expression of a wide variety of cell surface molecules. Although there is variance, suppressive T-regs have similar expression of surface molecules including CD4,CD25, and FOXP3. FOXP3 is a transcription factor that is crucial for both the maturation and function of cells, and is regarded the defining molecule for certain lineage (Albany *et al* ., 2019).

FOXP3 is an intracellular protein that serves as a functional marker on regulatory T cells. These cells, also known as T-reg cells, are a component of the immune system and play a crucial role in preventing the development of autoimmune diseases. T-reg cells can manifest in several forms, such as CD4, CD25, and FOXP3. CD4, CD25, and FOXP3 are widely recognized as the most prevalent phenotype for T-reg cells (Abid and Alwan.,2016).FOXP3 is a distinctive indicator of regulatory T cells (T-regs) and plays a crucial role in regulating the growth and function of T-regs. Without FOXP3, T-reg function is lost, leading to severe autoimmune disorders in humans(Wang *et al* ., 2023b).

T-reg FOXP3 Related with Ischemic Heart Diseases

T-reg Foxp 3 related with cardiovascular diseases T-regs are involved in cardiovascular disease, similar to their function in other autoimmune conditions. One reason for the weak connection between T-regs and cardiovascular illness may be that overall T-regs exhibit a wide range of antigen specificities, and it is possible that T-regs targeting antigens

related to atherosclerosis and cardiovascular disease are not prominent more accurate indicators of cardiovascular disease (Björkbacka.2016). T regulatory cells inhibit various immunological responses to self-antigens, contributing to the maintenance of peripheral tolerance by restricting autoimmune diseases and pathological immune reactions, including reducing immune response to oncoprotein encoded antigens. FOXP3 expression is essential for maintaining the stability of regulatory T cells and influences their functional activity. Genetic variations in the master controller FOXP3 and its associated elements have been associated with autoimmune disorders in humans, including IPEX. T-reg employs multiple immunosuppressive mechanisms to restrain an immune response by targeting effector cells. These mechanisms include secretion of immunoregulatory cytokines , granzyme / perforinmediated cell cytolysis, metabolic perturbation, influencing the maturation and function of antigen-presenting cells (APC), and releasing extracellular vesicles to promote immunological tolerance (Grover *et al* .,2021).Accumulating studies over decades indicate the critical involvement of immune cells and inflammatory cytokine production in the development of IHD including atherosclerosis and MI. While inhibiting inflammatory responses and targeting immune cells provide opportunities to impact ischemic myocardial injury(Zhuang and Feinberg.,2020).

2.6.2.2 Interleukin 35 (IL-35)

Interleukin 35 is a relatively new cytokine and part of the IL-12 cytokine family with significant roles in immune regulation and suppression (Ye *et al*.,2021). It was first identified in 2007 and has since been recognized for its unique immunosuppressive properties (Li *et al*.,2019). IL-35 is produced by T-regs and B-regs, which are essential for maintaining immune tolerance and preventing autoimmune responses (Yi et al.,2024). IL-35 is characterized by a heterodimeric structure that is unique among

biologically active cytokines Figure (2.4) It is composed of IL12p35 (α-subunit, encoded by IL12α) and Epstein–Barr virus–induced gene 3 (β-subunit, encoded by Ebi3 (Yi *et al.*,2024). IL-12α (p35), this subunit is shared with other cytokines in the IL-12 family, such as IL-12 itself ,IL-27β (EBI3) This subunit is also part of IL-27, another member of the IL-12 cytokine family(Huang *et al.*,2017).The unique structure of IL-35 allows it to interact with specific receptors on target cells. IL-35 can signal through the IL-12Rβ2 (part of the IL-12 receptor) and gp130 (part of the IL-27 receptor) chains12. Interestingly, IL-35 can signal through either of these receptor chains independently, which adds to its versatility in immune modulation (Li *et al.*,2019).

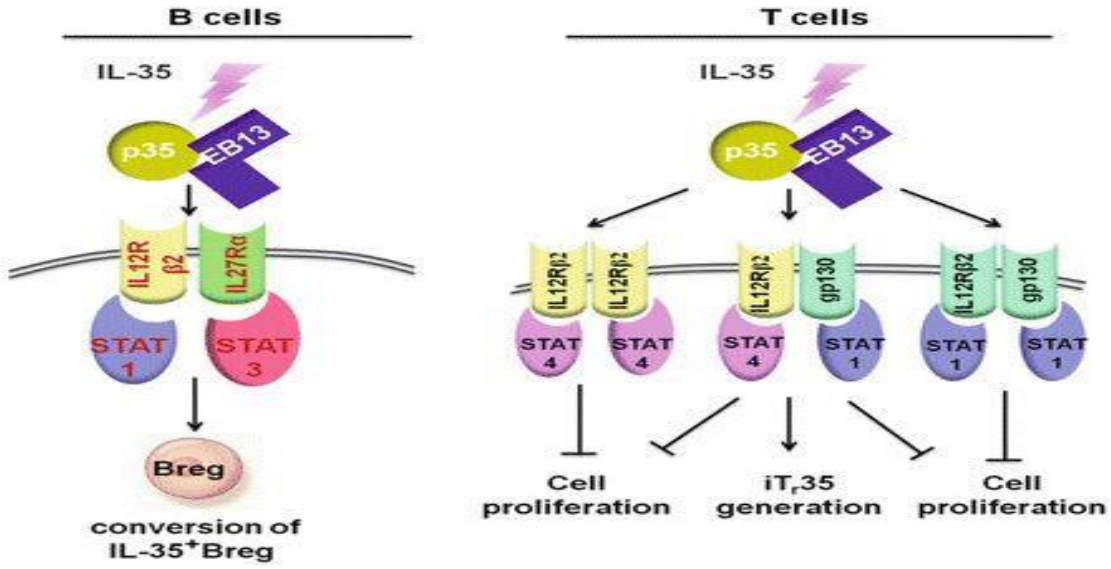


Figure (2.4) : Structure and Function of Interleukin-35 (Song and Ma, 2016).

IL-35 is a potent immunosuppressive cytokine that plays a crucial role in maintaining immune homeostasis. It achieves this by inhibiting the proliferation of effector T cells, particularly Th1 and Th17 cells12 (Liu *et al.*,2023). Th1 cells are involved in the response against intracellular pathogens, while Th17 cells play a role in autoimmune diseases (Damsker *et al.*,2010). IL-35 is known to promote the expansion of T-regs, which are crucial for maintaining immune tolerance and suppressing excessive immune responses. This expansion helps in preventing autoimmune diseases and maintaining immune homeostasis (Hao *et al.*,2018).

Role of IL-35 in Ischemic Heart Diseases

As a new member of the IL-12 family (Zhang and Xing. 2023). IL-35 regulates cardiovascular disease progression through immune homeostasis and inflammatory suppression.IL-35 deficiency or impaired function may be present in a variety of cardiovascular diseases, including atherosclerosis, acute coronary syndrome, pulmonary hypertension, abdominal aortic aneurysm, heart failure, myocardial ischemia–reperfusion, Aortic dissection and myocarditis (Feng and Wu.2022). IL-35 increases the suppressive capacity of the anti-inflammatory regulatory T cells (T-regs).and thus, suppresses the immune response. IL-35 plasma levels were shown to be decreased in patients suffering from AMI, unstable angina, and stable angina, compared to patients with chest pain syndrome (chest pain with no signs of cardiovascular diseases (Lin *et al*.,2012).In 2020 found that plasma IL-35 levels were significantly lower in patients with stable coronary artery disease than in healthy controls. Therefore, the researchers believe that IL-35 levels may be a new biomarker for stable CHD (Oflar *et al*.,2022).

2.6.2.3 Interleukin 17A (IL-17A)

Interleukin-17A is a pro-inflammatory cytokine signature cytokine secreted by T helper 17 (Th17) cells, which play an essential role in maintaining immune homeostasis and promoting immune dysregulation (Schinocca *et al*.,2021), IL-17A is one of the currently known six members of the IL-17 cytokine family (IL-17A, IL-17B, IL-17C, IL-17D, IL-17E and IL-17F) and is implicated in immune responses to infectious pathogens and in the pathogenesis of inflammatory autoimmune diseases (von *et al*.,2020). Interleukin-17A (IL-17A) is indeed a 155-amino acid protein that forms a disulfide-linked homodimer. This structure is crucial

for its function in the immune system. Each subunit of the homodimer has a molecular mass of approximately 15-20 kDa, and together, the homodimer has a total molecular mass of around 35 kDa (Kochhar *et al*.,2021).IL-17A is a secreted glycoprotein, meaning it has carbohydrate groups attached to it, which can influence its stability and interactions with other molecules. This cytokine plays a significant role in inflammatory and autoimmune responses, making it a key target for therapeutic interventions in diseases (Rex *et al*.,2023). The production of IL-17A begins with the differentiation of naive CD4+ T cells into Th17 cells. This process is driven by the presence of specific cytokines, particularly IL-6 and transforming growth factor-beta (TGF-β). These cytokines activate the transcription factor RORγt, which is crucial for the development of Th17 cells (Ge *et al.*,2020).Once Th17 cells are differentiated, the cytokine IL-23 plays a vital role in their expansion and stabilization. IL-23 binds to its receptor on Th17 cells, promoting the expression of IL-17A through the activation of STAT3 (Signal Transducer and Activator of Transcription 3) and further enhancing RORγt activity (Krueger *et al*.,2024).Activated Th17 cells produce and release IL-17A. This cytokine can then bind to its receptor, IL-17RA, on various cell types, leading to the production of other proinflammatory cytokines and chemokines, which recruit neutrophils and other immune cells to the site of inflammation (Rex *et al.*,2023). The production of IL-17A is tightly regulated to prevent excessive inflammation. Regulatory T cells (T-regs) and other anti-inflammatory cytokines, such as IL-10, help modulate the activity of Th17 cells and the production of IL-17A(Miossec .2017).

Role of IL-17A in Ischemic Heart Diseases

Interleukin-17A (IL-17A) is a pro-inflammatory cytokine and considered significant contributor to the development of CAD (Ghaznavi *et al*.,2020). elevated levels of IL-17A are associated with an increased risk of ischemic cardiovascular diseases (ICVD). that higher IL-17A levels contribute to the development of ICVD, suggesting IL-17A as a potential risk marke (Miao *et al.*,2024). Interleukin 17A (IL-17A) plays a significant role in the development and progression of IHD (Oliveira *et al*.,2021). IL-17A is involved in the inflammatory processes that contribute to atherosclerosis, a major underlying cause of ischemic heart disease. It promotes the recruitment of inflammatory cells to the vascular endothelium, which can lead to plaque formation and instability (Taleb and Tedgui.2018). Studies on cardiovascular diseases often find elevated levels of IL-17 in the blood serum of affected patients(Allam *et al.*, 2018).The concentration of serum IL-17A and the platelet aggregation levels were obviously elevated in ACS patients compared with stable angina patients (Băghină *et al.*,2024).

2.6.2.4 The high-sensitivity C-Reactive Protein (hs-CRP)

C-Reactive Protein is a type of protein, mainly produced by liver organ, it can be elevated in plasma patients with acute inflammation (Fu *et al.*, 2023). Structurally, CRP is a 206 amino acid cyclic pentameric protein with five identical subunits that are not covalently bonded, excessive body weight that has been associated with elevated CRP levels (Stanimirovic *et al*., 2022). Since fat cells secrete a variety of inflammatory molecules called adipocytes, including leptin, adiponectin, and others, obese people constantly have high levels of inflammation (Kim and Yeun, 2022). It is commonly but incorrectly believed that high-

sensitivity and conventional CRP are two different entities. The hs-CRP is a biochemical test which is a highly sensitive quantification of CRP. High sensitivity is a new modified assay which measures very low levels of CRP in plasma. It gives us an estimate of general levels of inflammation in body, giving an idea of the inflammatory status (Banait *et al*.,2022). Early elevation of serum hs-CRP levels is associated with a higher risk of developing CVD and mortality. hsCRP increase is also important in predicting the occurrence of CVD (Lee *et al*.,2023).that CRP might affect CAD progression through various pathways, such as activating the complement system and platelets, suppressing fibrinolysis, promoting the proliferation of smooth muscle cells, microphage polarization, and lipid deposition. Researchers have verified CRP functioning in vivo as driving inflammation, platelet aggregation, and thrombosis (Polyakova and Mikhaylov.2020).A higher risk of angina is related to a high baseline hs-CRP (Nath *et al*.,2023).hs-CRP level in patients with unstable angina were significantly higher than those in patients with stable angina ,the level of hs-CRP in patients with unstable angina is associated with the severity of coronary stenosis (Seyedian *et al*.,2016).People with greater baseline hs-CRP concentrations have a doubled risk of stroke and a tripled risk of MI (Alkado and Al-Helaly.,2022).hs-CRP may be a simple marker of the magnitude of the inflammatory response to myocardial ischemia .Besides myocardial necrosis and infarct size, other kinds of tissue damage could cause a hs-CRP elevation in patients with STEMI, such as atherosclerotic mass, underlying inflammatory process, and circulating proinflammatory cytokines .Hs-CRP has been shown associated with hospital outcomes: death, MI, and angina. This underlines the role of this biomarker in the risk assessment (Milano *et al*.,2019).

2.7 Diagnosis and Assessment of Ischemic Heart Diseases

A range of non-invasive and invasive tests of ischaemia and functional significance of coronary disease are clinically available (Demir *et al*., 2022).

2.7.1 Non Invasive Functional Test

Electrocardiogram (ECG) includes exercise stress test, Stress echocardiography (SE), Single-Photon Emission Computed Tomography (SPECT),Positron Emission Tomography (PET), Cardiovascular Magnetic Resonance (CMR) ,Cardiac Computed Tomography Angiography (CCTA) and Fractional flow reserve (FFR) (Baggiano *et al*.,2023).

2.7.1.1 Electrocardiogram(ECG)Exercise Stress Test

The electrocardiogram (ECG) is one of the most common diagnostic tools available to assess cardiovascular health. ECG is mainstay of medical practice due to their clinical relevance, low cost, and wide availability(Avula *et al*.,2023). The Bruce protocol is the most commonly used format. It starts the patient on a treadmill at a speed of 1.7 miles per hour and a 10% incline. Every 3 minutes, the speed and angle of incline are increased.An ECG stress test is considered diagnostic if the patient achieves at least 85% of the maximum age-predicted heart rate. If a test is terminated before achieving this threshold because of positive findings but the results meet the ECG criteria for ischemia, then the results are still considered positive for ischemia. However, if a test is terminated before achieving 85% of the predicted heart rate and there are no ECG changes, it is considered nondiagnostic as it is not known whether ischemic

changes would have occurred if the patient had continued to the required workload (Matta *et al* .,2021).

2.7.1.2 Exercise Stress Echocardiography (Echo)

is a well-validated and commonly used test for the evaluation of suspected coronary artery disease (CAD). A normal stress echocardiogram, defined as the absence of any left-ventricular wall motion abnormality at rest or with stress, portends an excellent prognosis. In contrast, exercise stress Echo that reveals regional wall motion abnormalities is specific for CAD (specificity 80%-88%) and is associated with an increased risk of adverse cardiac events (Daubert *et al*

.,2020).

2.7.1.3 Single Photon Emission Computed Tomography (SPECT)

the SPECT has also a high diagnostic value in coronary artery disease and has been proposed as a principal measure for patients(Javan *et al*.,2022).Nuclear medicine techniques have a great deal of advantage of using gamma radiation emitter radiolabeled compounds to diagnose the long list of infectious and malignant disorders in human systems. The gamma emitter radionuclide-labeled compounds are associated with SPECT camera. SPECT camera mainly offers the detection and analysis of gamma rays origin to furnish the imaging of defective organs in the body. There are about 85% radiopharmaceuticals in clinical practice which are being detected by SPECT camera (Naqvi and Imran. 2021) . injected into the patient's body. Intravenously administrated radiopharmaceuticals accumulate in specific body part or organ for which it is prepared and scans are obtained by SPECT camera . Scan generated by SPECT camera gives very fruitful information regarding disease (Payolla *et al*.,2019).

2.7.1.4 Positron Emission Tomography (PET)

is a powerful tool for the diagnosis of coronary heart disease. It has a higher diagnostic accuracy and the addition of quantitative information yields incremental prognostic value. Cardiac PET can comprehensively assess all aspects of coronary heart disease, from coronary atherosclerotic plaque to the myocardial tissue characterization(De Almeida *et al*., 2022).Positron emission tomography is a non-invasive, functional imaging test utilizing ionizing radiation, the source of which is a radioactive isotope (radionuclide) administered to the patient. By measuring the radioactivity in the examined organs, PET enables tracking dynamic biological processes in vivo on three-dimensional images. The principle of cardiac PET imaging is based on intravenous administration of a radionuclide-tagged tracer molecule (radiotracer) and subsequent registration of the radiation emitted by it by the detector in which the patient is placed(Blach *et al*.,2023).

2.7.1.5 Cardiovascular Magnetic Resonance (CMR)

enables assessment and quantification of morphological and functional parameters of the heart, including chamber size and function, diameters of the aorta and pulmonary arteries, flow and myocardial relaxation times(Kawel *et al*.,2020).Stress CMR can provide accurate assessment of myocardial ischemia, viability, and function. The absence of ionizing radiation with CMR combined with its high contrast and spatial resolution are advantageous, especially in younger or pregnant women. Stress CMR images are obtained with the use of vasodilators (typically adenosine, regadenoson or dipyridamole) to induce hyperemia followed by a gadolinium-based contrast agent injected peripherally; serial T1-weighted CMR images are then acquired. The contrast enters normally

perfused myocardial regions more quickly and in higher concentrations which can be detected as a greater increase in T1-signal relative to abnormally perfused regions . Although not widely available, CMR has the potential to quantify MBF and detect CMD defined by invasive coronary reactivity testing(Gaine *et al*.,2022).

2.7.1.6 Coronary Computed Tomography Angiography (CCTA)

is a non-invasive imaging modality with high sensitivity for the detection of coronary artery disease (CAD).the CCTA has role for management of patients with stable ischaemic heart disease. Available computed tomography (CT) technology allows the quantification of plaque burden, identification of high-risk plaques, or the functional assessment of coronary lesions for ischaemia detection and revascularization for refractory angina symptoms(Antonopoulos *et al*.,2022). CCTA has trans-formed the non-invasive assessment of CAD enabling visualisation of the coronary lumen, stenoses and plaque features, in three dimensions (3D). These data are now available at low X-ray exposure (often comparable with CACS), and in short acquisition times that reduce the need for breath-holding or beta-blocker treatment to slow the heart rate. Protocol optimisation however does remain heterogeneous between individual centres internationally (Channon *et al*.,2022).

2.7.1.7 Fractional Flow Reserve (FFR)

Fractional flow reserve (FFR) is an index to characterise the functional significance of coronary artery stenosis .Although FFR is computed as the ratio between invasively measured post-stenotic and central aortic pressures, this index was originally derived to represent the ratio between the actual transtenotic flow over the hypothetical flow that would be observed in the absence of the stenosis under examination (Müller *et*

al.,2021). FFR is defined as the ratio of maximum achievable blood flow through a blockage (area of stenosis) to the maximum achievable blood flow in the same vessel in the hypothetical absence of the blockage (Hill *et al.*,2022).FFR can be measured with a pressure wire during ICA to evaluate the hemodynamic significance of stenosis. An FFR value of ≤ 0.80, has excellent diagnostic accuracy (> 90%) for identifying coronary stenosis that causes myocardial ischemia(Yun *et al*.,2021).

2.7.2 Invasive Functional Test

The goal of invasive coronary angiography is to identify the cause of a patient's symptoms that are possibly related to myocardial ischaemia (De Bruyne *et al*.,2023) .Coronary angiography is understood as the visualization of the coronary arteries through the injection of a radiopaque contrast material and recorded in radiographic images in digital form. This technique is now the most reliable way to identify coronary anatomy and pathology for therapeutic decision making in patients with myocardial ischemia .The images obtained of the coronary arteries by angiography only show the vascular lumen, seen in a two-plane projection. This allows the cardiologist to assess and measure the magnitude of the coronary lesions according to the degree of stenosis of the vascular lumen in relation to the "healthy" reference segments (Escudero *et al*.,2021).

Chapter Three
Materials and Methods

3.1 Instruments and Equipment's:

The equipment's and instruments used in this study were listed in the tables

Table (3.1): Instruments that used in the current study.

NO	Tools	Company	Country
1	Cotton	Voltaren	China
2	Gloves	Voltaren	China
3	Masks	Voltaren	China
4	Tourniquet	Voltaren	China
5	Disposable Syringe	Jangsu	China
6	Gel tube	Hightop	China
7	Test Tube Rack	Citotest	China
8	Eppendrof Tubes	Appendrofs tubes	China
9	Eppendrof Tube Rack	Citotest	China
10	Pipette Tips	Gilson	U.S.A
11	EDTA tube	Hightop	China

Table (3.2): Equipment's that used in the current study.

NO	Tools	Company	Country
1	Centrifuge	Kokusan H-19F	Japan
2	Deep freeze	Ateko	Denmark
3	ELISA reader	Human	Germany
4	ELISA washer	Human	Germany
5	Micropipette	Slammed	Germany
6	spectrophotometer	Human	Germany

3.2 Prepared Kits :

The commercial kits used in the present study are shown in table

Table (3.3): commercial kits used in the study

NO	Types of kits	Company	Country
1	Human CPP (Copeptin) ELISA Kit	Elabscience	U.S.A
2	Human hs-CRP (high-sensitivity C-Reactive Protein) ELISA Kit	Elabscience	U.S.A
3	Human TNNI3/cTn-I (Troponin I Type 3 ،Cardiac) ELISA Kit	Elabscience	U.S.A
4	Human VWF (Von Willebrand Factor) ELISA Kit	Elkbiotech	U.S.A
5	Human FOXP3 (Forkhead Box Protein P3) ELISA Kit	Elabscience	U.S.A
6	Human IL-35 (Interleukin 35) ELISA Kit	Elabscience	U.S.A
7	Human IL-17A (Interleukin 17A) ELISA Kit	Elabscience	U.S.A
8	Cholesterol Kit	Randox	U.K
9	HDL Kit	Randox	U.K
10	Triglyceride Kit	Randox	U.K
11	HBA1c Kit	Linear	Spain

3.3 Subjects

This study was carried out for IHD patients at Imam AL Hasan Al Mujtaba Hospital and Karbala Center for Cardiac Diseases and Surgery at the Imam Hussain Medical Educational Hospital in karbala province All the patients that included in the current study were diagnosed by Cardiologist. This study was conducted during the period from March 2024 to August 2024.

All participants were included in this study divided into Two main group with age range 25-85 years as fallowing:

- Group 1 Control
- Group 2 Patients divided into four subgroups:
    A. A.With risk factors (Hypertension, diabetes mellitus, obesity ,smoking).
    B. Stable angina
    C. Unstable angina
    D. Myocardial infraction

3.4 Inclusion and Exclusion Criteria:

3.4.1 Inclusion Criteria:

1. Patients with ischemic heart diseases.
2. Patients aged 25-80 years.
3. Patient with hypertensions , Diabetes Mellitus, Obesity , Smoking.
4. Patient with Complete data .

3.4.2 Exclusion Criteria:

1. Patients age less than 25 year.
2. Patients with cancer.

3. Patients with Autoimmune diseases.
4. Patients with renal failure.
5. Patients with Asthma.
6. Patient with Incomplete data .

3.5 Ethical Approval

Ethical approval was recorded according to Ethical Committee at the College of Applied Medical Sciences/University of Kerbala. Also ،the study achieves the permission of research ethics in the Ministry of Health of Iraq/Karbala health department ,the Imam AL Hasan Al Mujtaba Hospital and Karbala Center for Cardiac Diseases and Surgery at the Imam Hussain Medical Educational Hospital.The study objectives were described to all participants and verbal approvals were obtained from them.

3.6 Samples Collection:

Five milliliters of venous blood were drawn from both Patients with IHD and patients without IHD using a five-milliliter disposable syringe.The blood sample was immediately transformed into gel tube. Then, it was centrifuge from 5000 rpm for 5 min period to isolate serum , then serum was put into nine Eppendorf tubes (for estimating the level of troponin, copeptin , High sensitivity CRP, VWP , Treg Foxp3 ,IL17,IL-35, Lipid Profile) and kept in the deep freezer (-20 ˚C) until analysis, and used the EDTA tubes for HBA1c analysis.

3.7 Study Design

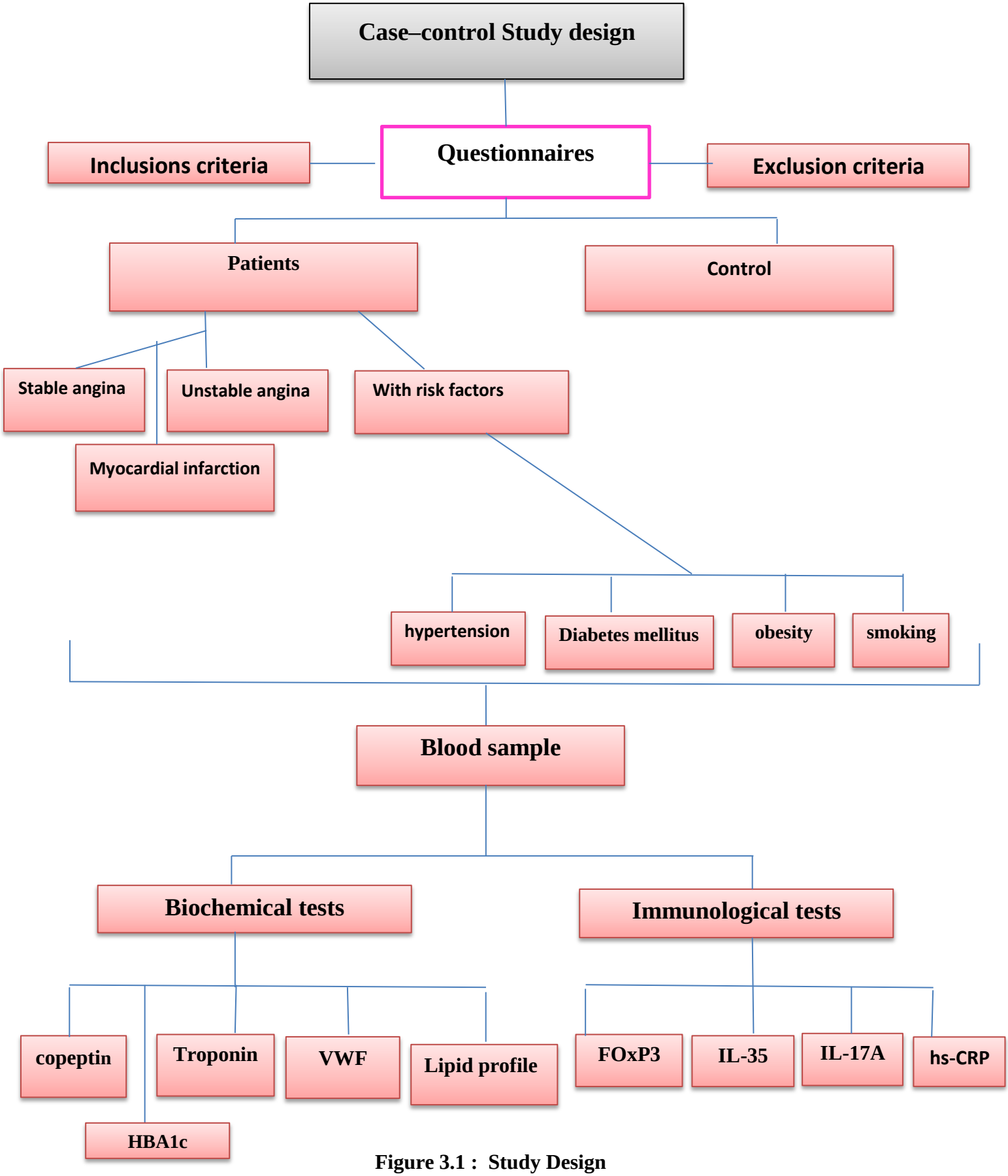


Figure 3.1 : Study Design

3.8 Biochemical Tests

3.8.1 Troponin I

- **Principle of the Test**

This ELISA kit use the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Human TNNI3/cTn-I. Samples (or Standards) were added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human Troponin I Type 3, Cardiac (TNNI3/cTn-I),TNNI3/cTn-I and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components were washed away. The substrate solution is added to each well. Only those wells that contain Human Troponin I Type 3, Cardiac (TNNI3/cTn-I), biotinylated detection antibody and Avidin-HRP conjugate appeared blue in color. The enzyme substrate reaction was terminated by the addition of stop solution and the color turns yellow .The optical density (OD) was measured spectrophot ometrically at a wavelength of 450 ± 2 nm . The OD value was proportional to the concentration of Human TNNI3/cTn-I. can calculated the concentration of Human TNNI3/cTn-I in the samples by comparing the OD of the samples to the standard curve**.**

- **Assay Procedure**

1. Determine wells for diluted standard, blank and sample. Hundred microliter was added to each dilution of standard, blank and sample into the appropriate wells (It was recommended that all samples and standards be assayed in duplicate. It was recommended to determine the dilution

ratio of samples through preliminary experiments or technical support recommendations). the plate covered with the sealer provided in the kit, incubated for 90 min at 37℃.

2. The liquid decant from each well, do not wash. Immediately add 100 μL of Biotinylated Detection Ab working solution to each well. Cover the plate with a new sealer. Incubate for 1 hour at 37°C.

3. Decant the solution from each well ,three hundred fifty microliter was of wash buffer added to each well ,Soaked for 1 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times.

4. Hundred microliter of HRP conjugate working solution added to each well. the plate covered with a new sealer, and incubated for 30 min at 37°C.

5. The solution decanted from each well, wash process repeated for 5 times as conducted in step 3.

6. Ninety microliter of substrate reagent added to each well,the plate covered with a new sealer, and incubated for about 15 min at 37°C. The plate protected from light.

7. Fifty microliter of Stop Solution added to each well.

8.The optical density (OD) was determined of each well at once with a micro-plate reader set to 450nm.

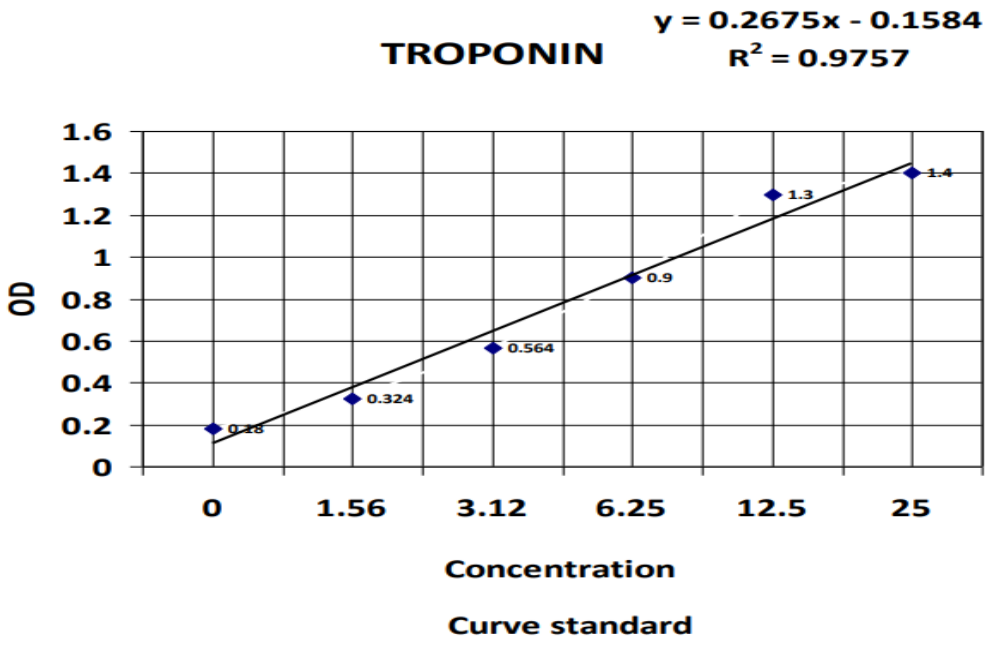


Figure3.2: Standard Curve for Troponin I

3.8.2 Copeptin

- **Principle of the Test**

This ELISA kit use the Competitive-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with Human CPP. During the reaction, Human CPP in samples or Standard competes with a fixed amount of Human CPP on the solid phase supporter for sites on the Biotinylated Detection Ab specific to Human CPP. Excess conjugate and unbound sample or standard were washed from the plate, and Avidin conjugated to Horseradish Peroxidase (HRP) were added to each microplate well and incubated. Then a TMB substrate solution was added to each well. The enzyme-substrate reaction is terminated by the added of stop solution and the color change was measured spectrophotometrically at a wavelength of 450 ± 2 nm. The concentration of Human CPP in the

samples was then determined by comparing the OD of the samples to the standard curve.

- **Assay Procedure**

1. Determine wells for diluted standard, blank and sample. Fifty microliter was added to each dilution of standard, blank and sample into the appropriate wells (It is recommended that all samples and standards be assayed in duplicate. It was recommended to determine the dilution ratio of samples through preliminary experiments or technical support recommendations). Fifty microliter of Biotinylated Detection Ab working solution was added to each well. The plate covered with the sealer provided in the kit ,incubated for 45 min at 37°C.

2.The solution decanted from each well, three hundred and fifty microliter added of wash buffer to each well ,soaked for 1 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. The wash was repeated 3 times.

3. Hundred microliter of HRP Conjugated working solution added to each well .The plate covered with a new sealer. Incubated for 30 min at 37°C.

4.The solution decanted from each well, wash process repeated for 5 times as conducted in step 2.

5. Ninety microliter of substrate reagent added to each well,the plate was covered with a new sealer, incubated for about 15 min at 37°C. The plate protected from light.

6. Fifty microliter of stop solution added to each well.

7. The optical density (OD value) was determined of each well at once with a micro-plate reader set to 450 nm

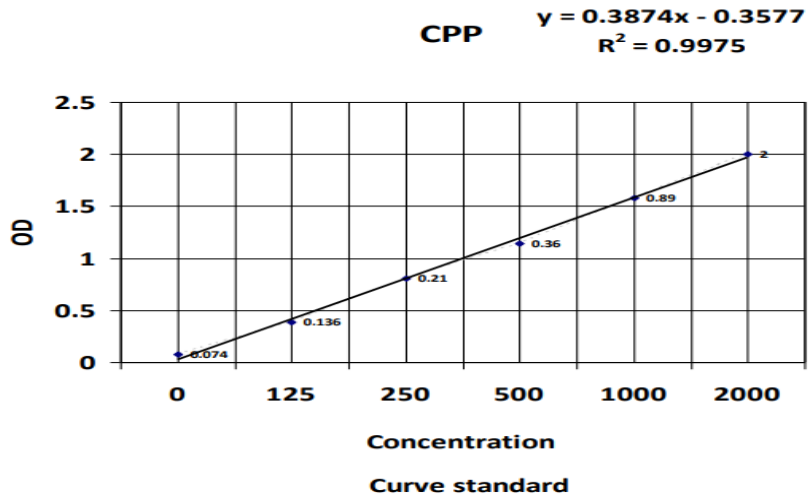


Figure3.3: Standard Curve for Copeptin

3.8.3 Von Willebrand Factor

- **Principle of the Test**

The test principle applied in this kit was Sandwich enzyme immunoassay. The microtiter plate provided in this kit had been pre-coated with an antibody specific to Human VWF. Standards or samples were added to the appropriate microtiter plate wells then with a biotin-conjugated antibody specific to Human VWF. Next, Avidin conjugated to Horseradish Peroxidase (HRP) was added to each microplate well and incubated. After TMB substrate solution was added, only those wells that contain Human VWF, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction was terminated by the addition of sulphuric acid solution and the color change was measured spectrophotometrically at a wavelength of

450nm ± 10nm. The concentration of Human VWF in the samples was then determined by comparing the OD of the samples to the standard curve.

- **Assay Procedure**

1. Determine wells for Diluted Standard, Blank and Sample. Seven wells Prepared for Standard, 1 well for Blank. Hundred microliter was added to each of standard working solution,or 100 μL of samples into the appropriate wells ,the Plate was covered. Incubated for 80 minutes at 37°C.

2. The liquid poured out of each well. The solution aspirated and washed with 200 μL of 1× Wash Solution to each well and let it sited for 1-2 minutes. the remaining liquid removed from all wells completely by snapped the plate onto absorbent paper. Totally washed 3 times. After the last wash, any remaining Wash Buffer was removed by aspirated or decanted. the plate inverted and blotted it against absorbent paper.

3. hundred microliter of biotinylated antibody working solution was added to each well, covered the wells with the plate covered and incubated for 50 minutes at 37°C.

4. The aspiration repeated ,washed process for total 3 times as conducted in step 2.

5. hundred microliter of Streptavidin-HRP Working Solution added to each well ,The wells covered with the plate sealer, and incubated for 50 minutes at 37°C.

6. The aspiration repeated, washed process for total 5 times as conducted in step 2.

7. Ninety microliter of TMB Substrate Solution added to each well, plate covered. Incubated for 20 minutes at 37°C in the dark. The liquid will turn blue by the addition of TMB Substrate Solution. Preheat the microplate readered for about 15 minutes before OD measurement.

8. Fifty microliter of stop reagent was added to each well. addicted of stop reagent the liquid will turn yellow. Mixed the liquid by tapping the side of the plate. If the color change was not uniform, gently tap the plate to ensure thorough mixed. The insertion order of the Stop Reagent should be the same as that of the TMB Substrate Solution.

9. Drop of water and fingerprint on the bottom of the plate wiped off and confirm there was no bubble on the surface of the liquid. Then, run the microplate reader and conducted measurement at 450 nm immediately.

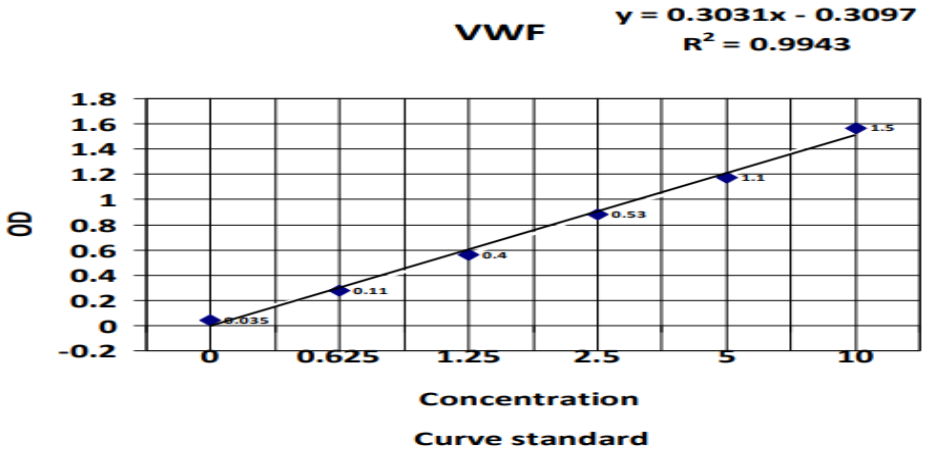


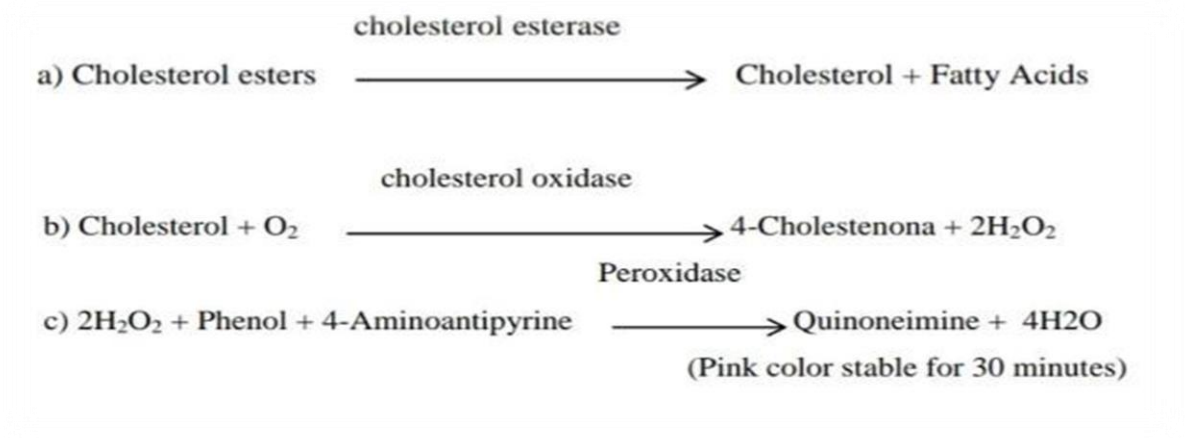
Figure3.4: Standard Curve for Von willbrand Factors

3.8.4 Lipid Profile

3.8.4.1 Determination of Serum Total Cholesterol (TC)

- **Principle**

The serum (TC) was measured using the enzymatic technique.
Cholesterol creates a colored complex within the specimen, as shown in the following:



The color intensity of the specimen was correlated with its cholesterol concentration. The red quinoneimine dye can be detected spectrophotometrically at 540 nm through an increase in absorbance.

- **Procedure (Manual)**

1 Every tube has 1.0 mL of reagent, and then preheated to 37 ° C for five

Min

2.0.01mL was added to the tube sample and mixed.

3. The tubes were incubated at 37 °C for five minutes .

4. Set the spectrophotometer to zero at 520 nm.

5. Absorbance was read for the whole trial.

- Calculation of Total Cholesterol (TC)

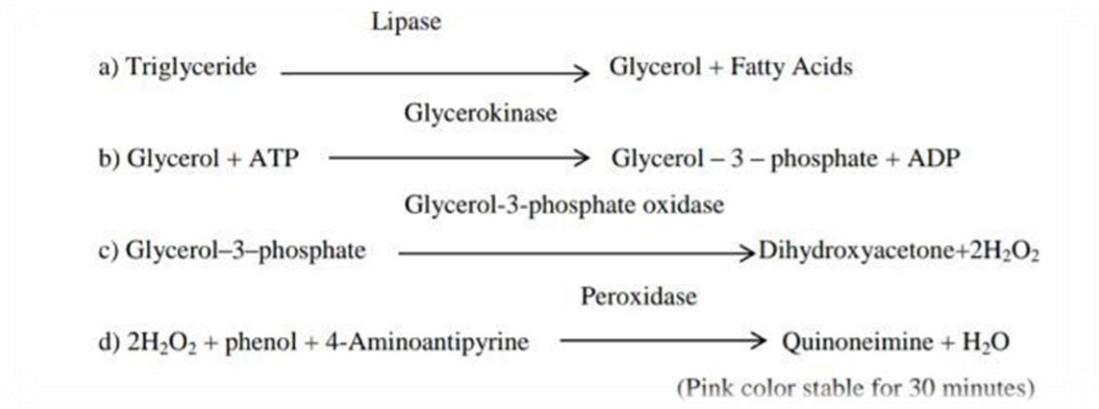
$$\text{Cholesterol (mg/dL)} = \frac{\text{(Abs. sample)}}{\text{(Abs. standard)}} \times \text{(Conc. of standard)}$$

Conc. of standard= (200 mg/dL)

3.8.4.2 Determination of Serum Triglyceride TG

- **Principle**

Incubating TG samples with lipoprotein lipase (LPL) results in the release of glycerin and free fatty acids . Glycerin is transformed into glycerin-3-phosphate (G3P) and adenosine-5-diphosphate (ADP) by glycerol kinase and ATP. Glycerin-3-phosphate (G3P) is, then, transformed into dihydroxyacetone phosphate (DAP) and hydrogen peroxide (H_2O_2) by glycerol phosphate dehydrogenase (GPO). In the final reaction, hydrogen peroxide (H_2O_2) reacts with 4 aminophenazone (4-AP) and p-chlorophenol in the existence of peroxidase to form quinoneimine.



The TG found in the product sample is a colored complex,

- **Calculation of Triglycerides**

$$\text{Triglycerides (mg/dL)} = \frac{\text{(Abs sample)}}{\text{(Abs standard}} \times \text{(Conc. of standard)}$$

Conc. of standard= (197mg/dL)

3.8.4.3 Determination of Serum High-Density Lipoprotein HDL

- **Principle**

Serum HDL was measured by the (HDL Kite Spinreact, Spin) using the enzymatic method, in which the chylomicron and lipoprotein of the very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) contained in the sample were precipitated by the addition of phosphotungestic acid in the presence of magnesium ions. The supernatant obtained after centrifugation contains HDL, from which cholesterol can be measured using the cholesterol enzymatic reaction.

- **Procedure**

Separation of HDL

1. 0.5 mL (500 µL) sample was added into respective tubes.

2. 0.05 mL (50 µL) reagent was added to each tube and mixed.

3. The tube stand was left for five minutes at room temperature.

4. The tube was mixed with and centrifuge at 1000-2000 g for at least 10 minutes

HDL Determination

1. Pipette 1.0 mL enzymatic cholesterol reagent was added to each tube.

2. Pipette 0.05 mL (50 µL) stander or clear supernatant (from step 4 above) to respective tubes.

3. All tubes were incubated for 10 minutes at 37°C.

4. Spectrophotometers were Zero with reagent blank at 500 nm.

5. All test tubes were read and recorded the absorbance.

- **Calculation of HDL**

$$\text{HDL (mg/dL)} = \frac{\text{(Abs. sample)}}{\text{(Abs. standard)}} \times \text{(Conc. of standard)}$$

Conc. of standard = (200mg/dL)

3.8.4.4 Determination of Serum Very Low Density Lipoprotein (VLDL)

Very low density lipoprotein can be determined from triglycerides divided by 5

VLDL = TG ÷ 5

3.8.4.5 Determination of Serum Low Density Lipoprotein (LDL)

Can be determined indirectly by using the Friedewald equation.

LDL = Total Cholesterol - (HDL + VLDL)

3.8.5 HBA1c Test

- **Principle**

The present procedure utilizes a weak binding cation-exchange resin for the rapid separation of glycated hemoglobin A1c from all the other hemoglobins. A hemolyzed preparation of the whole blood is mixed continuously for 5 minutes with a weak binding cation-exchange resin. During this time, HbA0 binds to the resin. HbA0 consist of all the other hemoglobins except A1c which remains in solution. After the mixing period, a filter was used to separated the supernatant containing the A1c from the resin. The percent glycohemoglobin is determined by measured the absorbance at 415 nm of the A1c fraction and the total hemoglobin fraction. The ratio of the two absorbances gives the percent of HbA1c.

- **Composition**

R1 Resin: Cation-exchange resin 8 mg/ml, buffered at pH 6.9. 3155105 25 x 2 mL; 3155110 100 x 2 mL

R2 Lysing solution: Potassium cyanide 10 mM, surfactant. 3155105 1 x 12,5 mL; 3155110 4 x 12,5 mL. R: 26/27/28

Standar. Calibrator: Liophylized, HbA1c 10%.

Filter separators: 3155105 25 filters; 3155110 100 filters.

- **Procedure**

Allow reagents to reach working temperature before using.

Hemolysate Preparation:

1. Five hundred microliter dispense Lysing Reagent (Reagent 2) into tubes labeled: Standard, Control, Sample 1, etc.

2. hundred microliter placed of the well-mixed blood sample, standard or control into the appropriately labeled tube, and mixed well.

3. It was allowed to stand for 5 minutes.

Glycohemoglobin preparation:

1. Seventy microliter of the hemolysate was added to the tube (Reagent 1).
2. The filter separators were positioned in the tubes so that the rubber sleeve was approximately 1 cm above the liquid level.
3. The tubes were placed on the rocker or rotator and mixed continuously for 5 minutes.
4. The tubes were removed from the rocker or rotator.
5. The Filter Separator was pushed into the tubes until the resin was firmly packed.
6. The supernatant was poured into another tube or directly into a cuvette for absorbance measurement.
7. The instrument was adjusted to zero absorbance at 415 nm with deionized water as the blank (Wavelength range: 390-420).

8. The absorbance values for the Standard, Control, Sample 1, etc., were read and recorded. These readings were for glycohemoglobin.

- **Calculation**

Results should be determined as follows:

$$\% \text{ HbA1c (unknown)} = \frac{\text{R (unknown)}}{\text{R (standard)}} \times \text{standard conc}$$

where:

$$\text{R (unknown)} = \text{Ratio (unknown)} = \frac{\text{Abs of HbA1c (unknown)}}{\text{Abs of Hb Tot (unknown)}}$$

$$\text{R (standard)} = \text{Ratio (standard)} = \frac{\text{Abs of HbA1c (standard)}}{\text{Abs of Hb Tot (standard)}}$$

3.9 Immunological Tests

3.9.1 Forkhead Box Protein P3 (FOXP3)

- **Principle of the Test**

This ELISA kit used the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Human FOXP3. Samples (or Standards) were added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human FOXP3 and AvidinHorseradish Peroxidase (HRP) conjugate were added successively to each micro plate

well and incubated. Free components were washed away. The substrate solution was added to each well. Only those wells that contain Human FOXP3, biotinylated detection antibody and Avidin-HRP conjugate will appeared blue in color. The enzyme-substrate reaction was terminated by the addition of stop solution and the color turns yellow. The optical density (OD) was measured spectrophotometrically at a wavelength of 450 ± 2 nm. The OD value was proportional to the concentration of Human FOXP3. calculated the concentration of Human FOXP3 in the samples by comparing the OD of the samples to the standard curve.

- **Assay Procedure**

1. The wells for the diluted standard, blank, and sample were determined. 100 µL of each dilution of the standard, blank, and sample were added to the appropriate wells (it was recommended that all samples and standards be assayed in duplicate). The dilution ratio of the samples was determined through preliminary experiments or based on technical support recommendations. The plate was covered with the provided sealer. Incubation was performed for 90 minutes at 37°C.
2. The liquid was decanted from each well without washing. 100 µL of Biotinylated Detection Antibody working solution was added to each well. The plate was covered with a new sealer. Incubation was performed for 1 hour at 37°C.
3. The solution was decanted from each well, and 350 µL of wash buffer was added to each well. The wells were soaked for 1 minute, then the solution was aspirated or decanted, and the wells were

patted dry against clean absorbent paper. This wash step was repeated 3 times.

4. Hundred microliter of HRP Conjugate working solution was added to each well. The plate was covered with a new sealer. Incubation was performed for 30 minutes at 37°C.
5. The solution was decanted from each well, and the wash process was repeated 5 times as described in step 3.
6. Ninety microliter of Substrate Reagent was added to each well. The plate was covered with a new sealer. Incubation was performed for about 15 minutes at 37°C, and the plate was protected from light.
7. Fifty microliter of Stop Solution was added to each well.
8. The optical density (OD value) of each well was determined immediately using a microplate reader set to 450 nm.

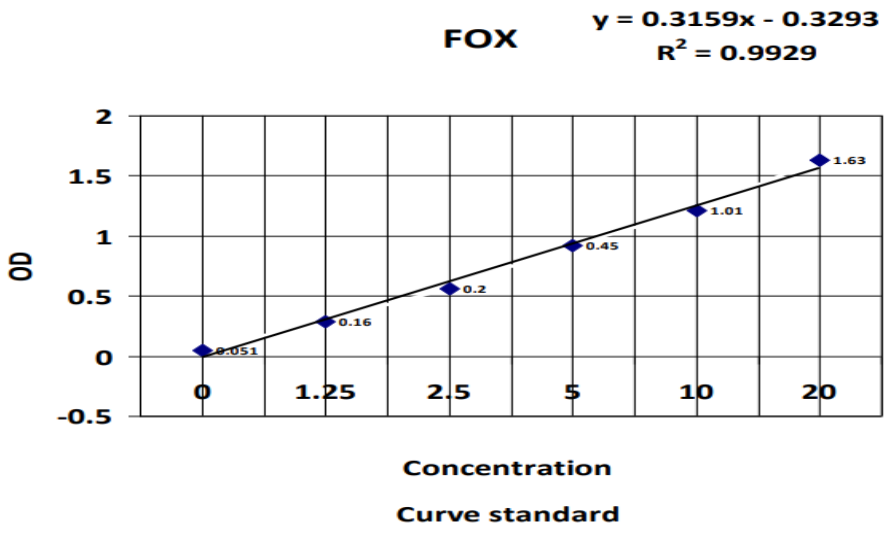


Figure3.5: Standard Curve for Foxp3

3.9.2 Interleukin-35 (IL-35)

- **Principle of the Test**

This ELISA kit used the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Human IL-35. Samples (or Standards) were added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human IL-35 and AvidinHorseradish Peroxidase (HRP) conjugate were added successively to each micro plate well and incubated. Free components were washed away. The substrate solution was added to each well. Only those wells that contain Human IL-35, biotinylated detection antibody and Avidin-HRP conjugate will appeared blue in color. The enzyme-substrate reaction was terminated by the addition of stop solution and the color turns yellow. The optical density (OD) was measured spectrophotometrically at a wavelength of 450 ± 2 nm. The OD value was proportional to the concentration of Human IL-35. we calculated the concentration of Human IL-35 in the samples by comparing the OD of the samples to the standard curve**.**

- **Assay Procedure**

1. The wells for the diluted standard, blank, and sample were determined. 100 μL of each dilution of the standard, blank, and sample were added to the appropriate wells (it was recommended that all samples and standards be assayed in duplicate). The dilution ratio of the samples was determined through preliminary experiments or technical support recommendations. The plate was

covered with the sealer provided in the kit. It was incubated for 90 minutes at 37°C.

2. The liquid was decanted from each well, without washing. Immediately, 100 µL of Biotinylated Detection Antibody working solution was added to each well. The plate was covered with a new sealer. It was incubated for 1 hour at 37°C.
3. The solution was decanted from each well, and 350 µL of wash buffer was added to each well. The wells were soaked for 1 minute, then the solution was aspirated or decanted, and the wells were patted dry against clean absorbent paper. This wash step was repeated 3 times.
4. Hundred microliter of HRP Conjugate working solution was added to each well. The plate was covered with a new sealer. It was incubated for 30 minutes at 37°C.
5. The solution was decanted from each well, and the wash process was repeated 5 times, as done in step 3.
6. Ninety microliter of Substrate Reagent was added to each well. The plate was covered with a new sealer. It was incubated for about 15 minutes at 37°C, and the plate was protected from light.
7. Fifty microliter of Stop Solution was added to each well.
8. The optical density (OD value) of each well was determined immediately with a microplate reader set to 450 nm.

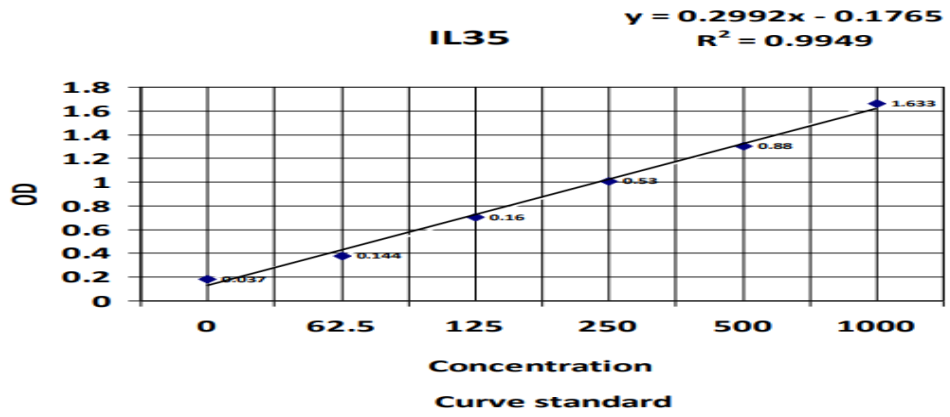


Figure3.6: Standard Curve for IL-35

3.9.3 Interleukin-17A (IL-17A)

- **Principle of the Test**

This ELISA kit used the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Human IL-17A. Samples (or Standards) were added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human IL-17A and AvidinHorseradish Peroxidase (HRP) conjugate were added successively to each micro plate well and incubated. Free components were washed away. The substrate solution was added to each well. Only those wells that contain Human IL-17A, biotinylated detection antibody and Avidin-HRP conjugate will appeared blue in color. The enzyme-substrate reaction was terminated by the addition of stop solution and the color turns yellow. The optical density (OD) was measured spectrophotometrically at a wavelength of 450 ± 2 nm. The OD value was proportional to the concentration of Human IL-17A. we calculated the concentration of Human IL-17A in the samples by comparing the OD of the samples to the standard curve.

- **Assay Procedure**

1. The wells for the diluted standard, blank, and sample were determined. 100 μL of each dilution of the standard, blank, and sample were added to the appropriate wells (it was recommended that all samples and standards be assayed in duplicate). The dilution ratio of the samples was determined through preliminary experiments or based on technical support recommendations. The plate was covered with the sealer provided in the kit. It was incubated for 90 minutes at 37°C.
2. The liquid was decanted from each well without washing. Immediately, 100 μL of Biotinylated Detection Antibody working solution was added to each well. The plate was covered with a new sealer. It was incubated for 1 hour at 37°C.
3. The solution was decanted from each well, and 350 μL of wash buffer was added to each well. The wells were soaked for 1 minute, then the solution was aspirated or decanted, and the wells were patted dry against clean absorbent paper. This wash step was repeated 3 times.
4. Hundred miroliter of HRP Conjugate working solution was added to each well. The plate was covered with a new sealer. It was incubated for 30 minutes at 37°C.
5. The solution was decanted from each well, and the wash process was repeated 5 times as performed in step 3.
6. Ninety miroliter of Substrate Reagent was added to each well. The plate was covered with a new sealer. It was incubated for about 15 minutes at 37°C, and the plate was protected from light.

7. Fifty microliter of Stop Solution was added to each well.
8. The optical density (OD value) of each well was determined immediately with a microplate reader set to 450 nm.

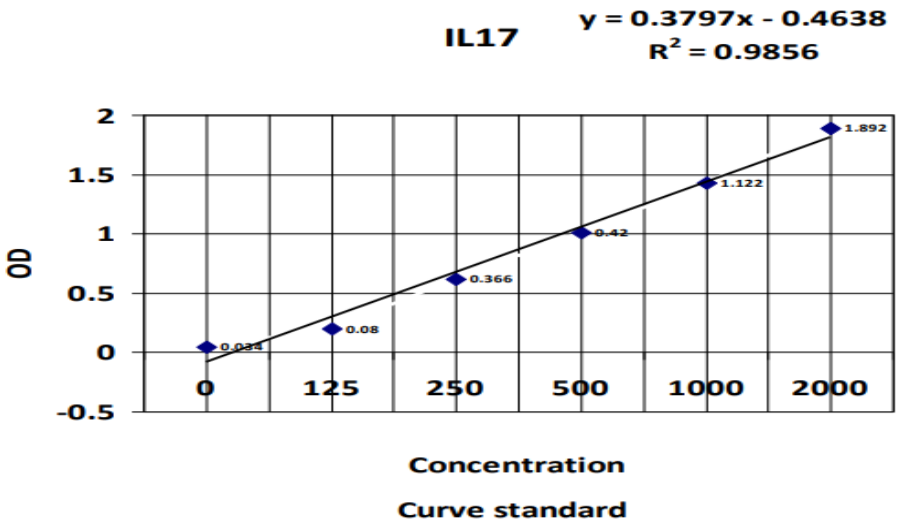


Figure3.7: Standard Curve for IL-17A

3.9.4 High-sensitivity C-Reactive Protein (hs-CRP)

- **Principle of the Test**

This ELISA kit used the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Human hs-CRP. Samples (or Standards) were added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human hs-CRP and AvidinHorseradish Peroxidase (HRP) conjugate were added successively to each micro plate well and incubated. Free components were washed away. The substrate solution was added to each well. Only those wells that contain Human

hs-CRP, biotinylated detected antibody and Avidin-HRP conjugate will appeared blue in color. The enzyme-substrate reaction was terminated by the addicted of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 ± 2 nm. The OD value was proportional to the concentration of Human hs-CRP.calculated the concentration of Human hs-CRP in the samples by comparing the OD of the samples to the standard curve**.**

- **Assay Procedure**

1. The wells for the diluted standard, blank, and sample were determined. 100 µL of each dilution of the standard, blank, and sample were added to the appropriate wells (it was recommended that all samples and standards be assayed in duplicate). The dilution ratio of the samples was determined through preliminary experiments or based on technical support recommendations. The plate was covered with the sealer provided in the kit. It was incubated for 90 minutes at 37°C.
2. The liquid was decanted from each well without washing. Immediately, 100 µL of Biotinylated Detection Antibody working solution was added to each well. The plate was covered with a new sealer. It was incubated for 1 hour at 37°C.
3. The solution was decanted from each well, and 350 µL of wash buffer was added to each well. The wells were soaked for 1 minute, then the solution was aspirated or decanted, and the wells were patted dry against clean absorbent paper. This wash step was repeated 3 times.

4. Hundred microliter of HRP Conjugate working solution was added to each well. The plate was covered with a new sealer. It was incubated for 30 minutes at 37°C.
5. The solution was decanted from each well, and the wash process was repeated 5 times as performed in step 3.
6. Ninety microliter of Substrate Reagent was added to each well. The plate was covered with a new sealer. It was incubated for about 15 minutes at 37°C, and the plate was protected from light.
7. Fifty microliter of Stop Solution was added to each well.

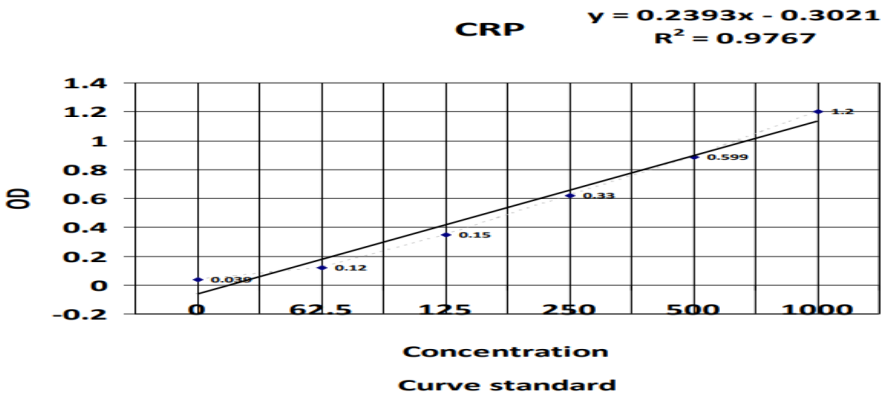


Figure3.8: Standard Curve for hs-CRP

3.10 Statistical analysis

The computer application SPSS used for statistical analysis of the data. The data were shown as mean standard deviation (SD). The results were deemed to have statistical significance since they were calculated differences between groups using the P value.

Chapter Four
Results and Discussion

4.1The Frequency of Groups with and without Ischemic Heart Diseases in Karbala Province

Sample distribution according to the severity of disease :The current study included 100 individuals with two main groups patients and control (85%, 15%) respectively as showed in Figure (4.1).

The diagram showed the percentage of patients (85%) which classified to with risk factors ,stable angina , unstable angina ,and myocardial infraction (25%,30% ,15%, 15%) respectively and showed 15% control group .

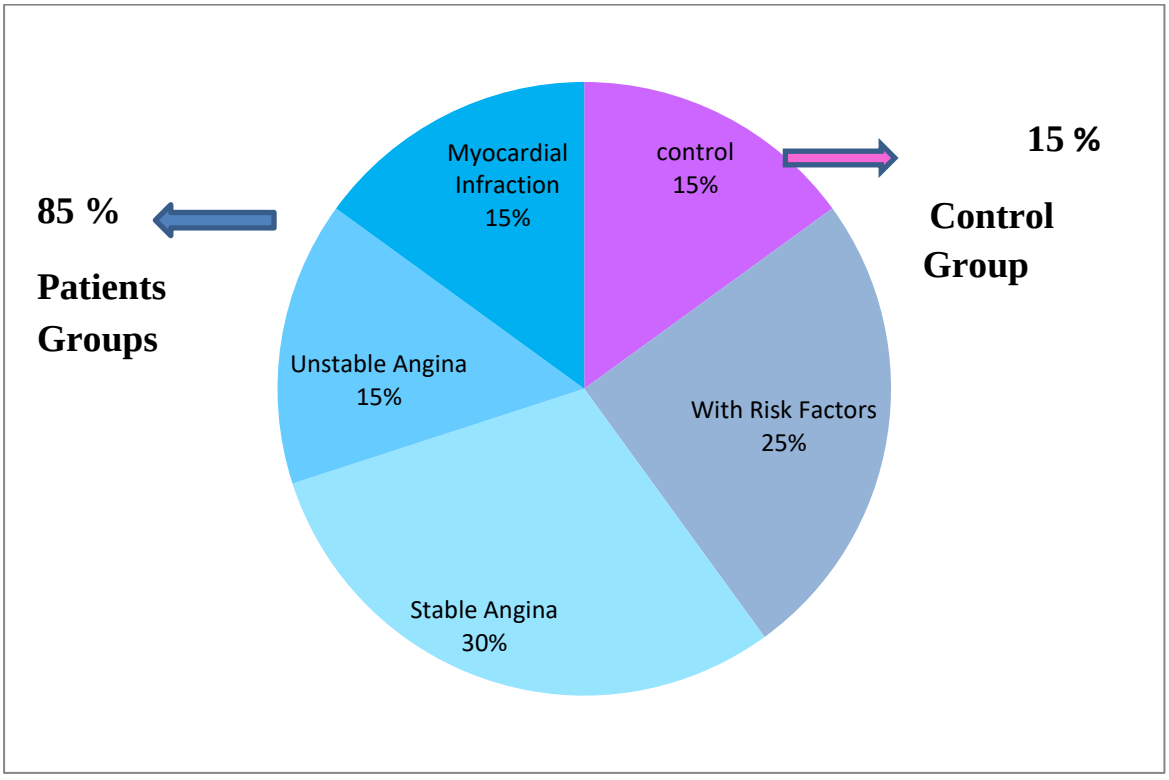


Figure (4.1) The frequency of groups with and without ischemic heart diseases in Karbala province .

4.2 Distribution and Characteristics of Groups with and without Ischemic Heart Diseases in Karbala Province

Based on the questionnaire the data obtained from the patients during the study period, it was found that the highest age group of patients was 60-69% (43.30%), while the samples were of both sexes, with a percentage of 53.30% for male and 46.70% for female, while the percentage of patients suffering from hypertension ,diabetes mellitus ,and smokers (88.30%,68.30%,and 78.30%) respectively in patients with ischemic heart disease.

Table4. 1: Distribution and Characteristics of groups with and without ischemic heart diseases According to the Risk Factor group (Age , sex , hypertension , diabetes mellitus , smoking)

Parameters	Level	Control	With Risk factor	Ischemic Heart Disease
Age Group	≤ 49	66.67%	52.00%	6.70%
	50 - 59	20.00%	36.00%	20.00%
	60 - 69	13.33%	8.00%	43.30%
	≥ 70	0.00%	4.00%	30.00%
Sex	Male	40.00%	40.00%	53.30%
	Female	60.00%	60.00%	46.70%
Hypertension	No	100.00%	44.00%	11.70%
	Yes	0.00%	56.00%	88.30%
Diabetes mellitus	No	100.00%	44.00%	31.70%
	Yes	0.00%	56.00%	68.30%
Smoking	No	100.00%	16.00%	21.70%
	Yes	0.00%	84.00%	78.30%

4.2.1 Body Mass Index (BMI)

In Figure (4.2) patients were stratified into normal weight, overweight, and obese groups ,in this study noticed most patients were normal weight group observe 11% ,1%,10% respectively. patients with overweight group showed results were 3%,4%,18% respectively ,while in patients in obese group the results showed 1% ,20% ,32% respectively (control ,with Risk factor, With ischemic heart diseases).

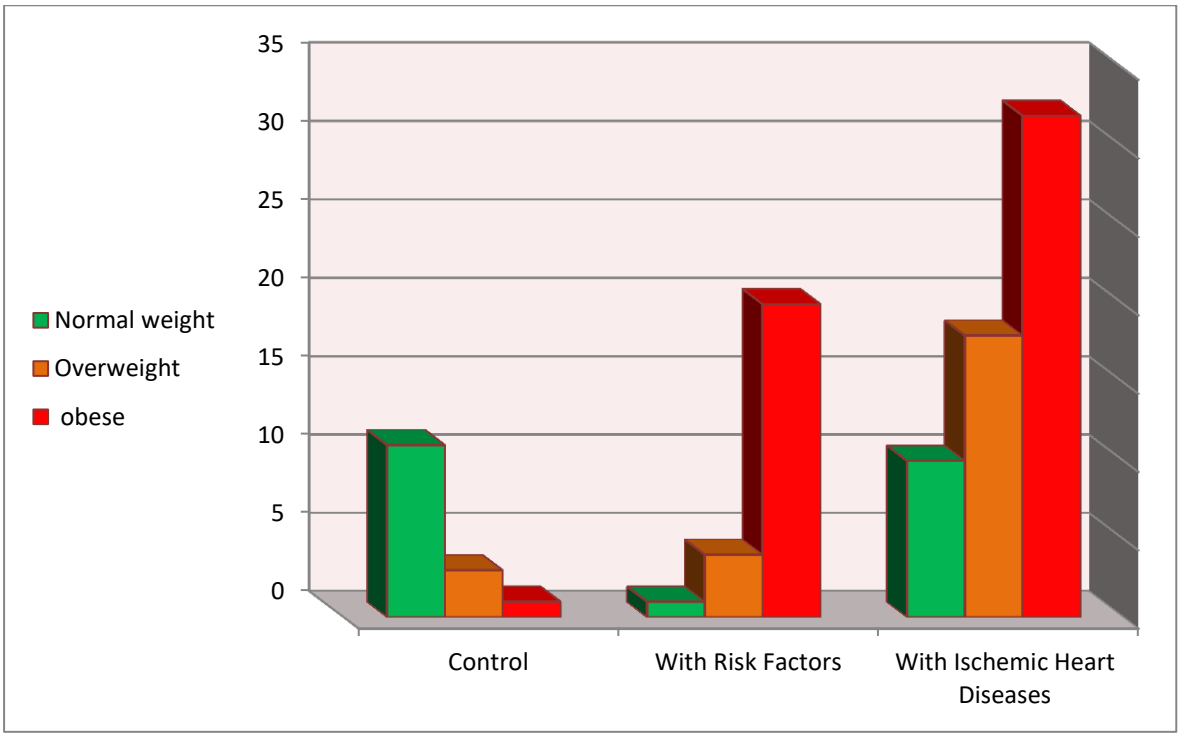


Figure (4.2): The frequency of groups with and without ischemic heart diseases According to the Risk Factors(BMI)

Obesity, assessed using body mass index (BMI) >30 kg/m2, is an established risk factor for development of coronary heart disease (CHD) in healthy individuals. Furthermore, obesity is associated with many of the known cardiovascular risk factors, such as hypertension and dyslipidemia(Held *et al.*,2022).Obesity has a strong related with coronary artery disease. Patients with a greater body mass index had more

advanced cardiovascular problems than those with a normal body mass index (Usmanov

.,2022).Higher BMI is associated with accelerated coronary atherosclerosis resulting in earlier presentation with symptomatic CAD. this suggesting that contributes to CAD through promotion of atherosclerosis (See *et al.*,2007).Patients with a higher BMI presented significantly more frequently with a history of stable angina (Atique *et al.*,2016).in other study found of increase in BMI resulted in a 49% increase in odds of unstable angina or myocardial infarction (Wolk *et al*.,2003).

4.3 Biochemical Tests

4.3.1 HbA1c

In the Table (4.3) the result showed increasing the concentration in patients with risk factors ,Stable angina , Unstable angina and Myocardial infraction (7.16±2.49, 7.83±2.52, 7.31± 1.69and 8.44±1.92) respectively. While the concentration in normal in group of control (4.62±0.44).

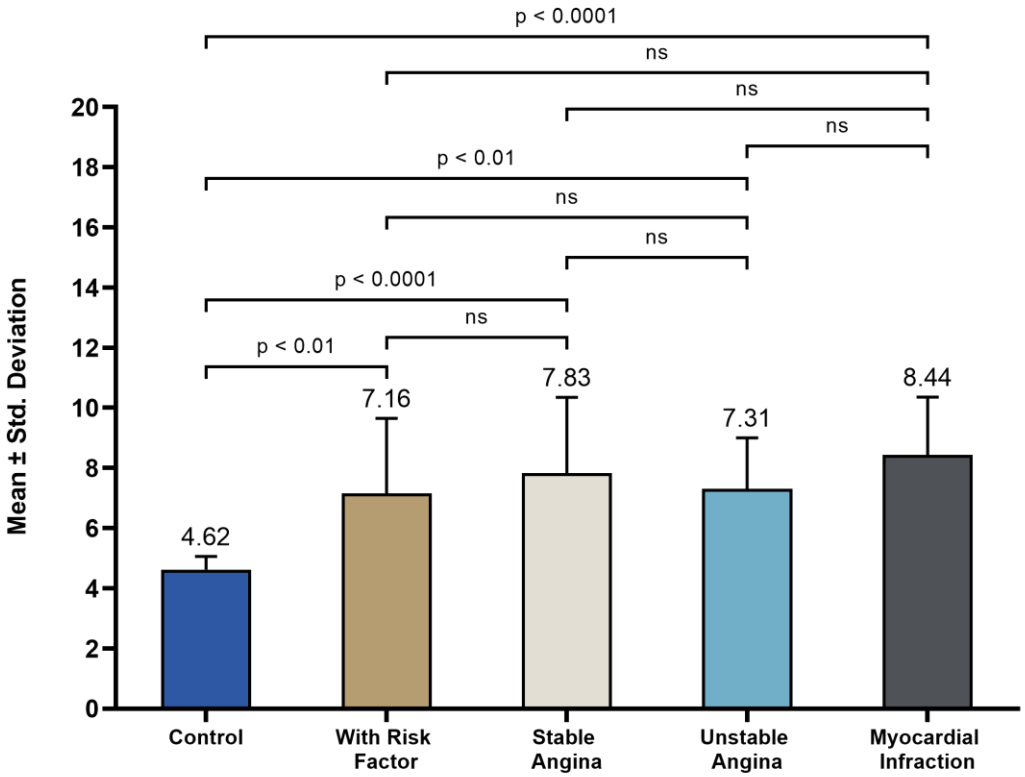


Figure (4.3): The Concentration of HbA1c In Patients With And Without Ischemic Heart Diseases.

This result agreement with study for Hong *et al*., (2014) showed high level of plasma HbA1C (>6.3%) was an independent predictor for the presence and severity of CAD as well as the early outcome of patients with stable angina, and agreement with other study for Butalia *et al*.,(2024). showed HbA1c ≥6.0% was associated with an increased risk of CVD and mortality outcomes. When blood glucose levels are high, glucose molecules attach to hemoglobin in red blood cells, forming glycated hemoglobin (HbA1c) (Eyth and Naik.2023). High levels of HbA1c indicate chronic hyperglycemia, which can lead to increased oxidative stress and inflammation, Oxidative stress and inflammation can damage the endothelium (inner lining of blood vessels), impairing its function and leading to atherosclerosis (build-up of plaques in arteries) (González *et al*.,2023).

4.3.2 Lipid Profile

The results in the table(4.2) showed increased in the concentration of cholesterol in with risk factors, stable angina, unstable angina and in myoardialinfraction (186.96± 45.18, 200.47 ±46.70 ,197.87 ±54.89 , 203 .53±5.89) respectively ,as compered with control (133 .80 ±16.37).

The results in the table(4.3) were indicated that increased in the concentration of Triglyceride in with risk factors, stable angina, unstable angina and in myoardial infraction (139.80± 60.90, 155.23 ±77.76 ,161.00±72.13, 162.13±59.39) respectively ,as compered with control (78.20±11.27).

The results in the table (4.4) were revelad that increased in the concentration of LDL in with risk factors, stable angina, unstable angina and in myoardial infraction(122.32 ±36.78 ,134.97 ±65.70 ,152.73 ±75.54 ,160.67±87.95) respectively ,as compered with control (66.40±14.60).

The results in the table (4.6) were showed that increased in the concentration of VLDL in with risk factors, stable angina, unstable angina and in myoardial infraction (27.96±12.18 ,31.04±15.58 ,32.20 ±14.43,32.43±11.88) respectively .as compered control (15.34±2.35).

The results in the table (4.5) were indicated that decreased in the concentration of HDL in control,with risk factors, stable angina, unstable angina and in myoardial infraction (37.80±7.56 ,42.26 ±9.54, 41.18±11.76, 43.79±10.13, 36.06±8.34) respectively.

Table (4.2): The concentration of Cholesterol in patients with and without Ischemic heart diseases groups

Level	Mean	Std. Deviation	P. value	Duncan Post hoc Test
Control	133.80	16.37	0.0001	a
With Risk Factor	186.96	45.18		b
Stable angina	200.47	46.70		b
Unstable angina	197.87	54.89		b
Myocardial infraction	203.53	55.04		b

Table (4.3): The concentration of Triglyceride in patients with and without Ischemic heart diseases groups

Level	Mean	Std. Deviation	P. value	Duncan Post hoc Test
Control	78.20	11.27	0.0020	a
With Risk Factor	139.80	60.90		b
Stable angina	155.23	77.76		b
Unstable angina	161.00	72.13		b
Myocardial infraction	162.13	59.39		b

Table (4.4): The concentration of LDL in patients with and without Ischemic heart diseases groups

Level	Mean	Std. Deviation	P. value	Duncan Post hoc Test
Control	66.40	14.60	0.0003	a
With Risk Factor	122.32	36.78		b
Stable angina	134.97	65.70		b
Unstable angina	152.73	75.54		b
Myocardial infraction	160.67	87.95		b

Table: (4.5) The concentration of HDL in patients with and without Ischemic heart diseases groups

Level	Mean	Std. Deviation	P. value	Duncan Post hoc Test
Control	37.80	7.56	0.1660	N/A
With Risk Factor	42.26	9.54		N/A
Stable angina	41.18	11.76		N/A
Unstable angina	43.79	10.13		N/A
Myocardial infraction	36.06	8.34		N/A

Table (4.6): The concentration of VLDL in patients with and without Ischemic heart diseases groups

Level	Mean	Std. Deviation	P. value	Duncan Post hoc Test
Control	15.34	2.35	0.0010	a
With Risk Factor	27.96	12.18		b
Stable angina	31.04	15.58		b
Unstable angina	32.20	14.43		b
Myocardial infraction	32.43	11.88		b

Current study was indicated that the results of triglycerides, cholesterol ,LDL an increased in angina, these results agreement with a study of Karantas *et al*., (2021) was indicated that angina may high levels of LDL cholesterol or triglycerides in the blood. Also ,this study revealed that Triglycerides, Cholesterol ,LDL an increased in myocardial infraction was consistent with Khan *et al*., (2013) study was observed significantly higher total cholesterol (TC) and triglyceride (TG) levels and levels in MI patients .The most common risk factors of CHD the are high levels of total cholesterol ,Triacylglycerol's (TAG), LDL, and VLDL (Alam *et al* .,2021).that elevated LDL triglycerides were associated robustly with an increased risk of ASCVD, ischemic heart disease, myocardial infarction, ischemic stroke, and peripheral artery disease (Balling *et al*.,2023).Elevated LDL cholesterol levels play a vital role in developing coronary artery disease. This occurs when LDL deposits in the coronary arteries form plaques and decrease blood supply to the heart muscle. Ischemia to the heart causes hypoxia, leading to chest pain (angina) and a heart attack (myocardial infarction) (Das and Ingole.2023).

4.3.3 Troponin I

The results in figure (4.4) showed the concentration of troponin I increased significantly ($p<0.00001$) in Myocardial infraction (0.066 ± 0.013ng/ml) compared with other parameters (control , with risk factors ,Stable angina ,unstable angina) (0.022±0.002, 0.019±0.004, 0.041 ± 0. 011, 0.039±0.005 ng/ml) respectively.

In another hand no increased significant between stable angina and unstable angina and may be the troponin as indicator in case myocardial infraction only.

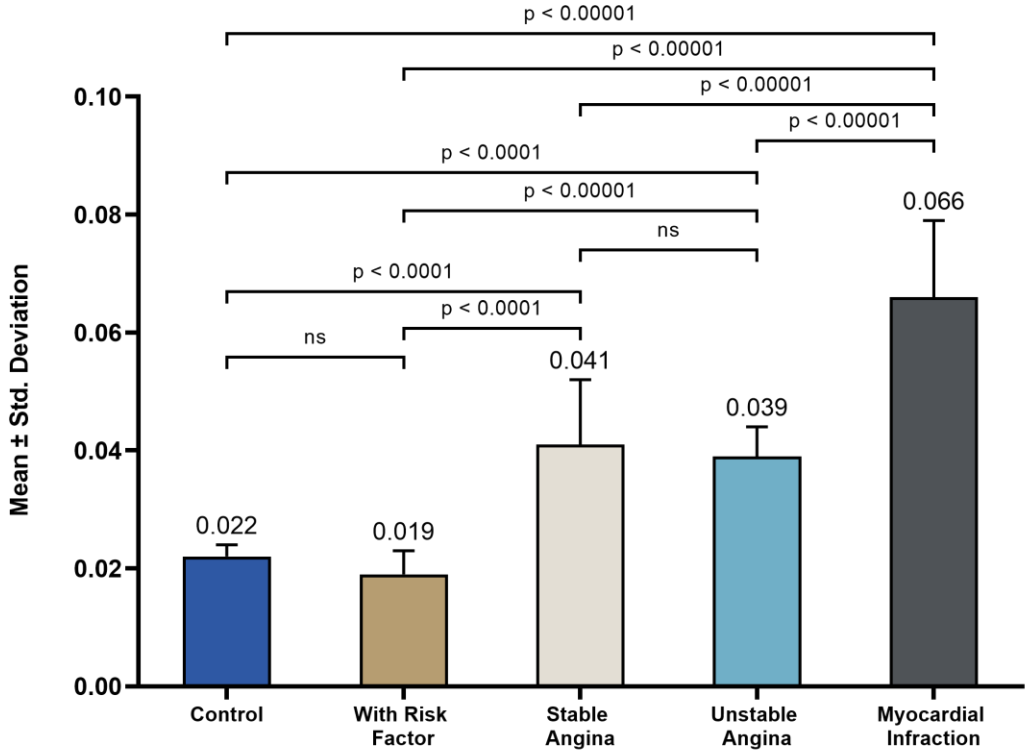


Figure (4.4) The Concentration of Troponin I in patients with and without Ischemic heart diseases.

This study was agreement with Januzzi *et al*., (2019) indicated that cardiac troponin I was elevated significantly in patients with myocardial infarction compared to healthy control ($p<0.0001$), and elevated concentrations of cTnI were associated with CAD. cTn I, with its high

specificity and sensitivity, has become the gold standard for diagnosing AMI (Chen *et al*.,2023). another study showed a slight no significant elevatied in serum Troponin I level in patients with stable angina (Danek *et al*.,2017), also, another study revealed that the level of troponin I was not significantly changed (0.0210±0.0034 vs 0.0200±0.0038 ng/ml, P = 0.054) in stable angina. However, in comparison with the healthy control (Hussein *et al*.,2023). This is because stable angina does not usually cause significant myocardial injury (Eggers *et al*.,2017). The cTn marker is synthesized and released from the normal myocardium (a healthy person has low serum levels and its level is increased during myocardial necrosis as in MI, it begins to rise 3 hours after the onset of chest pain. Its peak level reaches 18-24 hours after chest pain and remains elevated for 10 days(Aydin *et al*., 2019). So, cTnI is one of the criteria used in the diagnosis of MI. troponin is therefore mainly used in the emergency department as a rapid diagnostic test. when there is damage to the cardiac tissue, The cTnI are released into the blood stream and high circulating levels are used as indication for acute myocardial infarction (Cortes *et al*.,2024).

4.3.6 Copeptin

The results in figure (4.5) were showed the concentration of copeptin increased significantly ($p<0.0001$) in myocardial infraction (907.75±125.57 pg/mL) compared with other parameters(control ,with risk factors, Stable angina ,unstable angina (253.51±10.88 ,394.57 ± 101.96,425.18±105.53, 517.57±126.84 pg/ml) respectively.

Another hand the results showed a increased significant ($p<0.05$) between stable angina and unstable angina (425.18±105.53,517.57 ± 126 .84 pg/ml) respectively.

and no increased significant in copeptin between stable angina and group with risk factor (425.18±105.53,3 pg/ml) respectively.

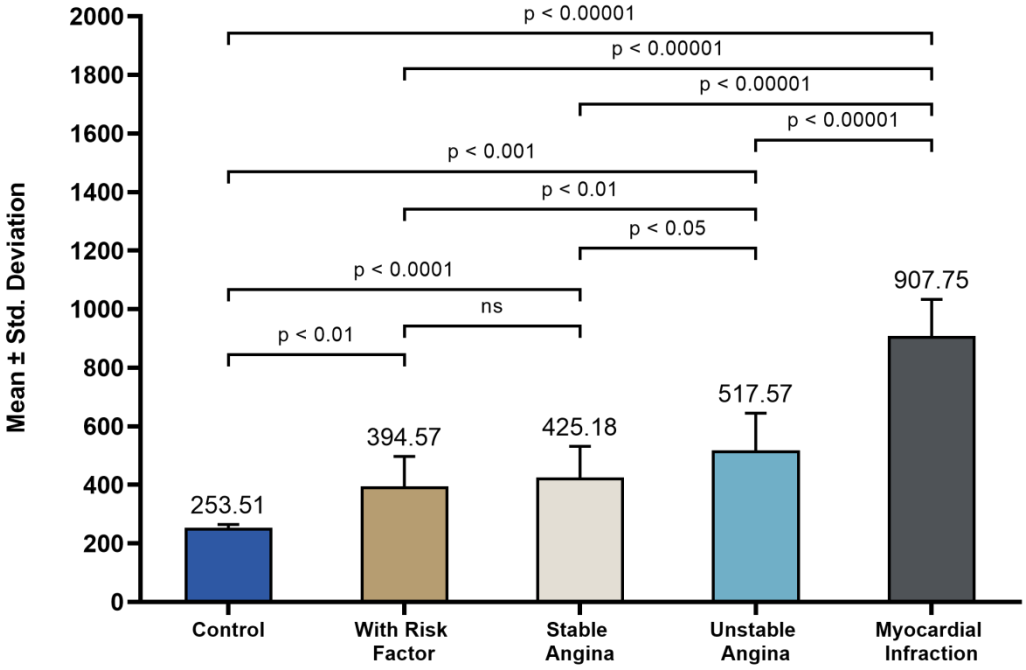


Figure (4.5):The Concentration of Copeptin in patients with and without Ischemic heart diseases.

current study was agreement with study of Allwsh and Aziz. (2015) they indicated the estimation copeptin levels in serum of heart diseases patients with compared individuals healthy. The results indicated that the normal level of copeptin in serum in control group .while there was a increased significant in copeptin concentration in patients of the heart disease compared with control group, and also This was consistent with the study Jeong *et al*., (2020) the results showed that elevated in serum copeptin increased significantly in MI compared unstable angina ($p<0.001$). Other study showed the levels of copeptin were increased significantly in the patients with myocardial infarction compared unstable angina, indicating that copeptin levels were strongly associated with the extent of myocardial necrosis (Mu *et al*.,2022), also proved the greater value of copeptin compared to troponin I for MI patients and its prognostic value for MACEs (cardiac death, re-infarction, re-

hospitalization for ischemic events, HF, stroke and TLR) and coronary revascularization within one year of follow-up ($p < 0.001$ for each) (Ahmed *et al*.,2021).Copeptin is a diagnostic and prognostic biomarker in CVD, including the rapid rule-out of acute myocardial infarction (AMI), mortality prediction in heart failure (HF), and stroke, the important role of the value of copeptin in the diagnosis, discrimination, and prognosis of CVD (AMI, HF, and stroke) (Mu *et al*.,2022). Copeptin is one of the biomarkers used in cardiovascular disease, as the C-terminal part of the pro-arginine vasopressin is secreted in equimolar quantities with it, and it also presents great stability in biological samples (Yildirim and Cabbar.,2019).In the case of AMI, copeptin is known to show increased values immediately, after which the values decrease until the third to fifth days after the event, when it reaches a plateau phase. It has been shown that it may be a good prognostic factor for MI, as well as for HF or ischemic stroke (Roczek *et al*.,2021).high plasma level of copeptin is associated with higher risks of mortality and MACEs in patients with CAD (Shi and Qian .2023). it is essential to realize that negative copeptin results do not indicate the absence of coronary artery disease, because the extent of copeptin release triggered by ischemia (such as unstable angina) was weaker than other acute stimuli, such as AMI(Keller *et al*.,2010).

4.3.7 Von Willbrand Factor (VWF)

The results in figure(4.6) were showed the concentration of VWF highly increased significantly ($p<0.00001$) in myocardial infraction (4.57 ± 0.99 ng/ml) compared with other parameters (control, with risk factors, Stable angina ,unstable angina) (0.24±0.16, 0.94±0.80, 1.44±0.65, 2.92± 0.35 ng/ml) respectively and also unstable angina (2.92 ng/ml) were increased significant ($p<0.00001$) compared with stable angina (1,44±0.65 ng/ml).

this results indicate the VWF as predictor because it increased significantly in unstable angina and continually highly increasing in myocardial infraction.

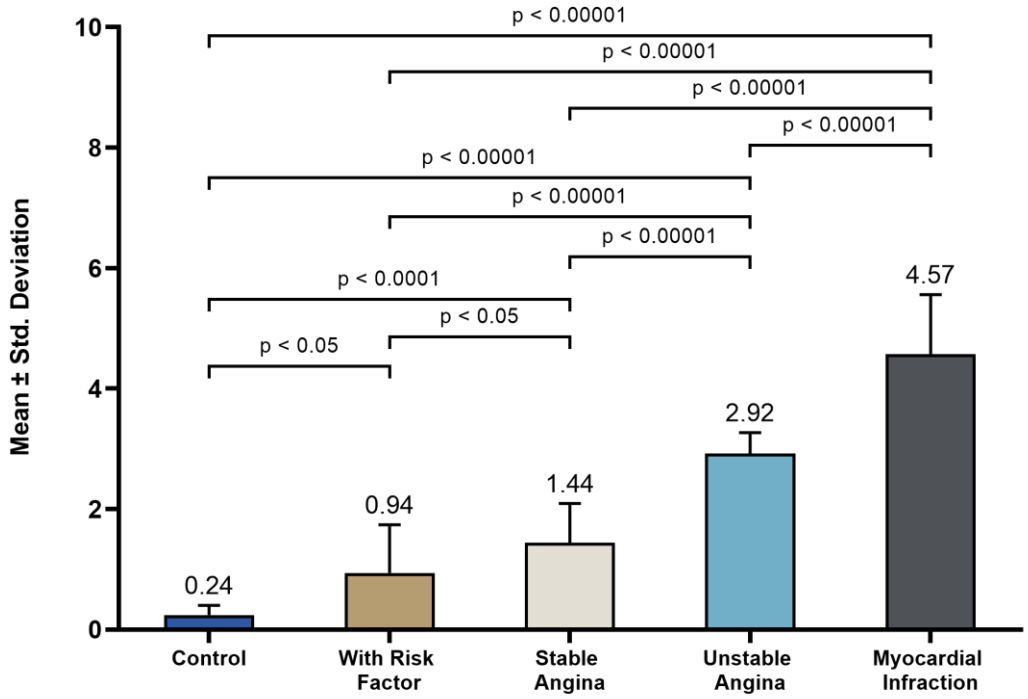


Figure (4.6) : The Concentration of Von Willbrand Factors in patients with and without Ischemic heart diseases.

This study was agreement with study for Morange *et al*., (2004) revealed that the baseline VWF levels were higher in patients who developed myocardial infraction than in control patients ,another study showed the level of VWF When compared to controls, patients with AMI had mean plasma VWF levels that were ~1.63 times higher ($p < 0.001$) (Xier *et al*.,2023). also, another study showed the levels of VWF in patients with AMI was significantly higher than that in the control group ($p < .001$) (Yan *et al*.,2020).The VWF is a plasma protein that mediates platelet adhesion and leukocyte recruitment to vascular injury sites and carries coagulation factor VIII, a building block of the intrinsic pathway of coagulation. The presence of ultra-large multimers of VWF in the bloodstream is associated with spontaneous thrombosis (Okhota *et*

al.,2020) .High concentrations of VWF in the blood are linked to well-known cardiovascular risk factors, such as advanced age, smoking, high cholesterol levels, diabetes mellitus, and hypertension. Moreover, elevated levels of VWF can predict the occurrence of stroke and vascular events in individuals with atrial fibrillation(Parvathareddy *et al*.,2024).VWF its dysfunction may contribute to the development of CAD and its complications (Kozlov *et al*.,2022).

4.4 Immunological Tests

4.4.1 High-sensitivity C-reactive protein (hs-CRP)

The results in figure (4.7) showed the concentration of hs-CRP highly increased significantly ($p<0.00001$) in myocardial infraction (7822.60 pg/ml) compared with other parameters (control, with risk factors, stable angina ,unstable angina (117.21±7.50, 249.77±74.30, 200.00 ± 53.12, 417.7±127.16 pg/ml) respectively and also unstable angina (417.75±127.16 pg/ml) was increased significant ($p<0.0001$) compared with stable angina (200.00±53.12 pg/ml) this result indicate hs-CRP as predictor because it increased significantly in unstable angina and continually highly increasing in myocardial infraction.

Another hand the results showed in patients with risk factor significant increase compared control ($p<0.001$) (249.77±74.30 , 117.21 ±7.50 pg/ml) respectively because this patients have another disease .

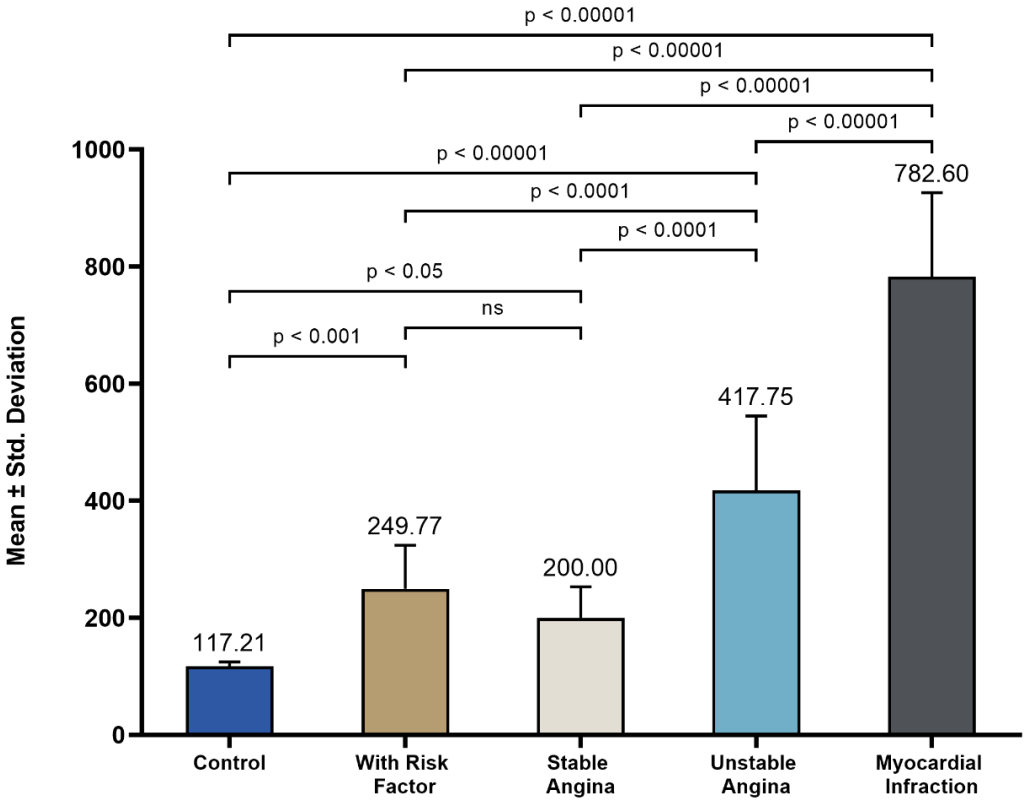


Figure (4.7) : The Concentration of high-sensitivity C-reactive protein (hs-CRP) in patients with and without Ischemic heart diseases .

This study was agreement with Oemrawsingh *et al*., (2016) they indicated Serum hs-CRP concentrations were dependent on the clinical presentation ($p<0.001$). Patients with stable angina pectoris (2.0 mg/L) had the lowest circulating CRP concentrations. Higher hs-CRP levels were observed in patients with unstable angina pectoris (5.0 mg/L) and patients with acute myocardial infarction (3.0 mg/L) ($p<0.001$), and other study revealed The mean levels of hs-CRP in the stable angina group, unstable angina group and the group with normal coronary angiography were 2.46 ± 1.79, 4.84 ± 3.38, and 2.95 ± 2.57 mg/L, respectively. The results showed that the mean levels of hs-CRP in patients with unstable angina was higher significantly compared to patients with stable angina ($P<0.050$) and normal patients (Seyedian *et al*.,2016). Serum hs-CRP levels are closely associated with an elevated risk of adverse cardiac events in individuals (Zhou *et al*.,2024).hs-CRP levels are recognized as a strong independent

risk marker for the identification of individuals at risk for future cardiovascular disease, which may be useful as an independent marker of prognosis for recurrent events in patients with stable coronary disease or ACS (Bouzidi *et al*.,2020). hs-CRP is produced by the liver in response to inflammation. Chronic inflammation can damage the inner lining of arteries, making them more susceptible to the buildup of fatty deposits (plaques) that narrow the arteries and restrict blood flow (Banait *et al*.,2022),and hs-CRP can contribute to the development of atherosclerosis, a condition where plaques form on the arterial walls. These plaques can rupture, leading to the formation of blood clots that can block blood flow to the heart, causing a heart attack(Nguyen *et al*.,2024). Patients with AMI, the level of hs-CRP may correspond to the extent of coronary artery lesion, the size of myocardial necrosis area, the risk of recurrent acute coronary syndrome, the risk of new-onset atrial fibrillation, ventricular tachycardia, heart failure decompensation / development, and death (Polyakova *et al*.,2020).

4.4.2 Forhkead Box P-3 (FOXP3)

The results in figure (4.8) showed the concentration of FOXP3 highly increased significantly ($p<0.00001$) in myocardial infraction (10.64± 1.15 ng/ml) compared with other parameters (control, with risk factors, stable angina ,unstable angina) (1.48±0.32, 2.83±1.03, 3.68±1.50, 5.51±1.04 ng/ml) respectively and also unstable angina (5.51± ng/ml) is increased significant ($p<0.001$) compared with stable angina (3.68±1.50 ng/ml) this result indicated the FOXP3 as indicator because it increased significantly in unstable angina and continually highly increased in myocardial infraction.

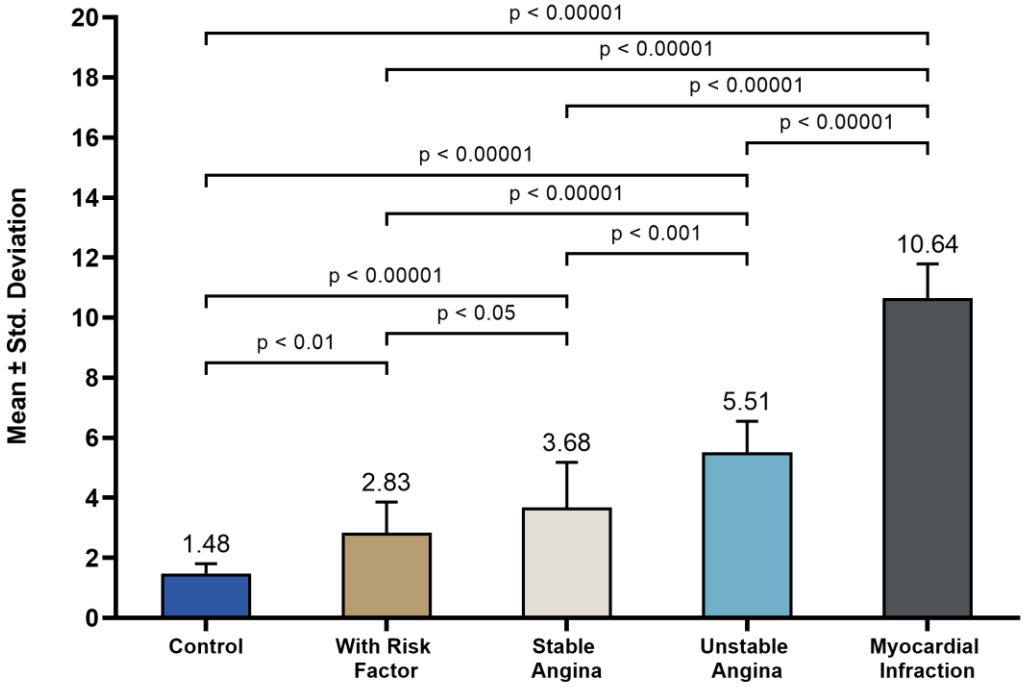


Figure (4.8) : The Concentration of FOXP3 in patients with and without Ischemic heart diseases .

Current study was agreement with Zhu *et al*., (2019) they revealed that elevated levels of FOXP3 are associated with increased risk for adverse outcomes in patients with ACS. Several studies have demonstrated recruitment of Tregs in the infarcted myocardium. has demonstrated increased levels of FOXP3, a transcription factor that critically regulates Treg function, in the infarcted myocardium (Dobaczewski *et al*.,2010).another study has suggested higher frequencies of circulating TregsFOXP3 in patients with stable atherosclerosis compared with healthy controls (Mailer *et al*., 2017). Human T regulatory cells (Tregs), which are hypothesized to have a role in the pathogenesis of atherosclerosis, are identified by the marker FOXP3. Tregs are thought to accomplish this by inhibiting pro-atherogenic Th1 and Th17 lymphocyte-mediated immune mechanisms, activating and moving dendritic cells in the direction of the plaque, suppressing inflammatory macrophages and preventing them from transforming into foam cells, and lastly, by lowering the activation of endothelial cells (Jackowska *et al*., 2019).Tregs

are required to maintain self-tolerance and dampen immunity by secreting the immunosuppressive cytokine IL-10, Transforming growth factor (TGF)-β, and by direct contact-inhibition of Teff cells (Foks *et al*., 2015).Tregs are found in human atherosclerotic lesions at a frequency of 1.2–3.9% of all CD3+ T cells (Wolf *et al*., 2020).

4.4.3 Interlukin-35 (IL-35)

The results in figure (4.9) showed the concentration of IL-35 highly decreased significantly ($p<0.00001$) in myocardial infraction (146.15 ± 40.16pg/ml) compared with group control (405.90 ±112.4 pg/ml) and gradually decreased significant with other parameter (with risk factor , Stable angina ,unstable angina (157.87±66.67, 160.96±52.51,160 .35 ± 43.41 pg/ml) respectively.

This results indicated the IL-35 as high concentration in healthy person and it decreased significantly in patients with risk factor and Ischemic heart disease (stable and unstable angina and Myocardial infraction).

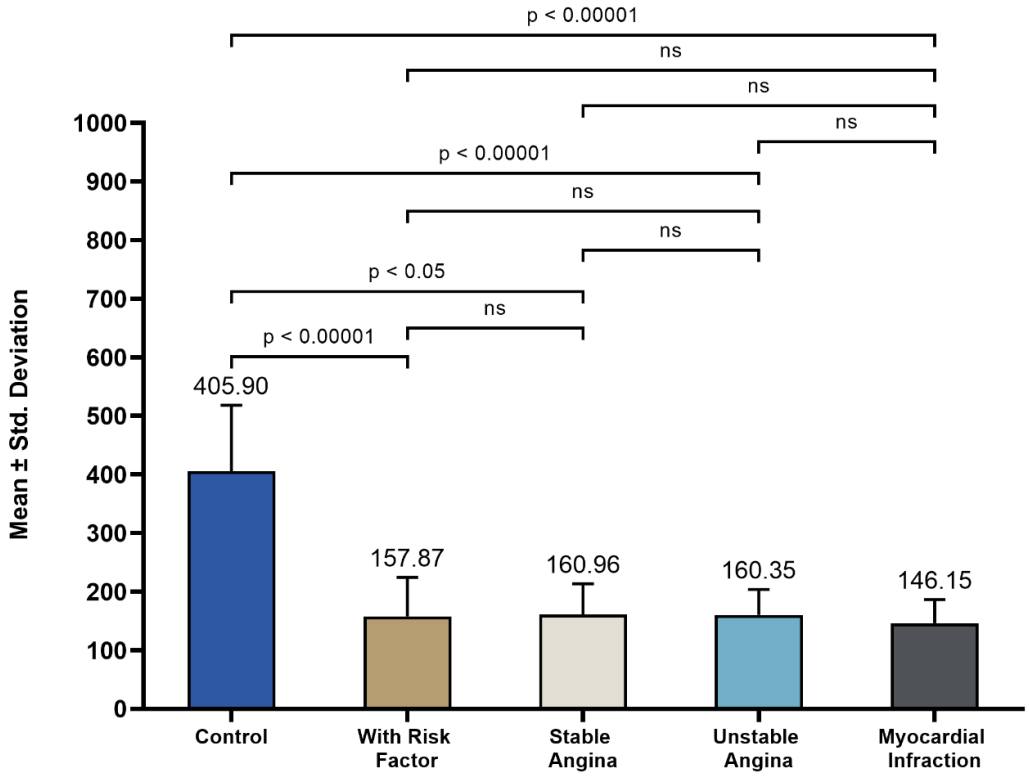


Figure (4.9) : The Concentration of interlukin-35(IL-35) in patients with and without Ischemic heart diseases .

This study was agreement with study by Li *et al*., (2019) that determined serum levels of IL-35 among ACS patients, involving (acute myocardial infarction and unstable angina), compared to control group , were indicated to be decreased significantly, compared with those in the control group. Another study showed that plasma IL-35 levels were decreased significantly in the stable angina group, the unstable angina group , and the AMI group compared with control group (Lin *et al*.,2012). Also ,another study showed the IL-35 levels of the CAD group were considerably lower compared to the control group ($p<0.008$) (Oflar *et al*.,2022). IL-35 a new member of the IL-12 family, is a cytokine secreted mainly by T-regs and B-regs, which inhibits inflammatory response and regulates immune homeostasis,that the occurrence and development of cardiovascular diseases are closely related to inflammatory response and immune homeostasis regulation, and they

have important role of IL-35 in this process (Feng and We .2022).Plasma level of IL-35 are closely related with coronary heart disease, which can be used as indicators of CHD evaluation and prognosis (Hu *et al*.,2017). that IL-35 is closely related to the occurrence and development of atherosclerosis as a very important potential cause of CHD (Zhang and Xing .2023). IL-35 influences the behavior of various immune cells, including T cells and macrophages. It promotes the development of regulatory T cells, which help maintain immune tolerance and reduce inflammation (Li *et al*.,2017).

4.4.4 Interlukin-17A (IL-17A)

The results in figure (4.10) showed the concentration of IL-17A highly increased significantly ($p<0.00001$) in myocardial infraction (944.38 ±172.02 pg/ml) compared with other parameters (control, with risk factors, Stable angina ,unstable angina (91.40±37.56, 333.70±87.24, 258.56±132.78,491.64±202.69 pg/ml) respectively and also unstable angina (491.64±202.69pg/ml) was increased significant($p<0.0001$) compared with stable angina (258.56±132.78 pg/ml) this result indicated the IL-17A as indicator because it increased significantly in unstable angina and continually highly increasing in myocardial infraction

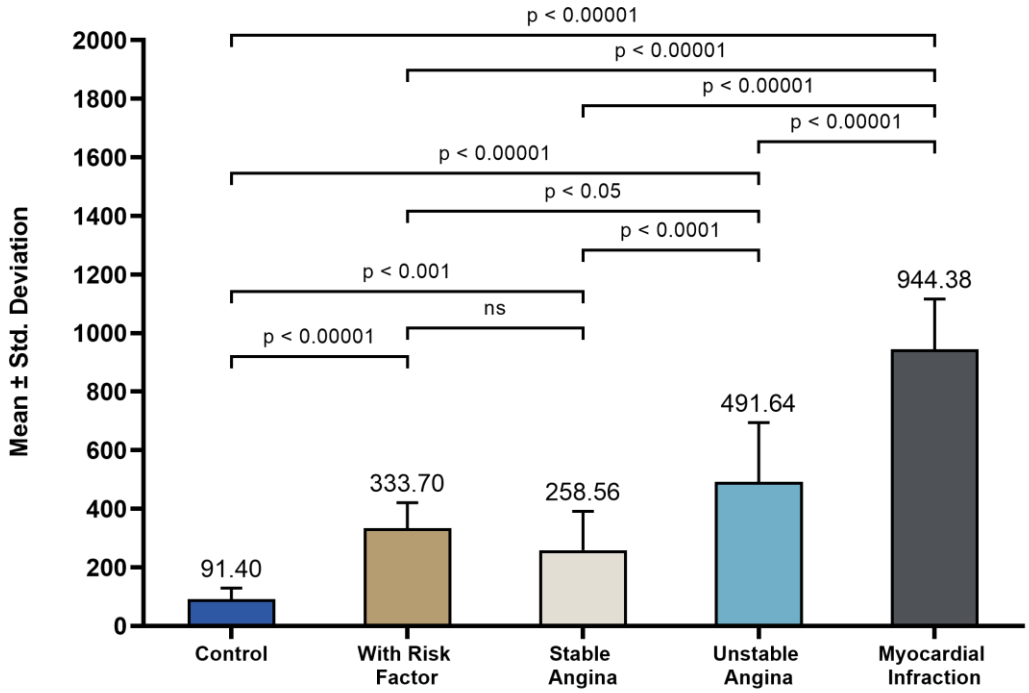


Figure (4.10) : The Concentration of interlukin-17A(IL-17A) in patients with and without Ischemic heart diseases.

This study was agreement with Miao *et al*.,(2024) that indicated IL-17 level were higher in the IHD group than those in the control group, and another study found Increased IL-17 expressing cells were found in plaques obtained from symptomatic patients (either stroke or transitory ischemic attack due to carotid stenosis(compared to asymptomatic patients (Erbel *et al*., 2014).also other study these findings point towards a role of inflammation in the form of increased activity of IL-17 in patients with unstable angina and acute myocardial infarction and thus suggested that IL-17 driven inflammation may play a role in the promotion of clinical instability in patients with coronary artery disease (Boluri *et al*.,2022). IL-17A plays a significant role in the development and progression of IHD ,IL-17A is a pro-inflammatory cytokine that contributes to chronic inflammation, which is a critical factor in the development of atherosclerosis, a major cause of IHD (Oliveira *et al*.,2021). IL_17A acts on vessel and cardiac cells, leading to inflammation, coagulation and thrombosis. Clinical studies and *in vivo*

have shown its involvement in the pathogenesis of cardiovascular diseases including atherosclerosis and myocardial infarction that occur prematurely in chronic inflammatory disorders (Robert and Miossec.2017).IL-17A mediates the endothelial inflammatory response, promotes water and sodium retention, and changes the electrophysiological structure of the atrium, accelerating the progression of ischemic stroke risk factors such as atherosclerotic plaques, hypertension, and atrial fibrillation (Nordlohne and Von.2019).

4.5 Correlation Coefficient Among Research Parameters

This table (4.7) indicated that the correlation coefficients among various research parameters in an all-patient group. Pearson and Spearman's correlation tests were used to assess the strength and direction of the association between the continuous variables. Below was a detailed explanation of the table:

- ❖ Troponin:

-Showed significant positive correlations with:

- VWF (R = 0.687, P = 0.001**)
- TregFoxp3 (R = 0.695, P = 0.007**)
- High CRP (R = 0.729, P = 0.004**)
- Copeptin (R = 0.799, P = 0.002**)
- IL-17 (R = 0.595, P = 0.005**)

- ❖ VWF:

-Showed strong positive correlations with:

- TregFoxp3 (R = 0.860, P = 0.001**)
- High CRP (R = 0.903, P = 0.006**)
- Copeptin (R = 0.819, P = 0.001**)
- IL-17 (R = 0.794, P = 0.003**)

❖ TregFoxp3:

-Correlated positively with:

- High CRP (R = 0.845, P = 0.002**)
- Copeptin (R = 0.861, P = 0.001**)
- IL-17 (R = 0.809, P = 0.005**)

❖ Hs-CRP:

-Strong positive correlations observed with:

- Copeptin (R = 0.845, P = 0.002**)
- IL-17 (R = 0.871, P = 0.001**)

❖ Copeptin:

-Showed a significant positive correlation with IL-17 (R = 0.847, P = 0.001**)

❖ IL-35:

-The results showed no significant correlations with most parameters, except a weak negative correlation with IL-17 (R = -0.092, P = 0.486, non-significant).

Table 4.7: Patients Correlation Coefficient Among Research Parameters according to the **all-patient groups**

Parameters	Value	VWF	TregFoxp3	High CRP	Copeptin	IL-35	IL-17
Troponin	R value	0.687**	0.695**	0.729**	0.799**	0.025	0.595**
	P value	0.001	0.007	0.004	0.002	0.848	0.005
VWF	R value		0.860**	0.903**	0.819**	0.014	0.794**
	P value		0.001	0.006	0.001	0.916	0.003
TregFoxp3	R value			0.845**	0.861**	-0.168	0.809**
	P value			0.002	0.001	0.198	0.005
High CRP	R value				0.845**	-0.024	0.871**
	P value				0.002	0.856	0.001
Copeptin	R value					0.004	0.847**
	P value					0.978	0.001
IL-35	R value						-0.092
	P value						0.486

-Pearson and Spearman's correlations were performed to assess the association strength and direction between the two continuous variables.

-**. Correlation is significant at the 0.01 level.

-*. Correlation is significant at the 0.05 level.

R-value: Indicates the correlation coefficient, showing the strength and direction of the relationship. Positive values indicate a direct relationship, while negative values indicate an inverse relationship.

P-value: Indicates the significance of the correlation .

- A p-value ≤ 0.01 (**) suggests a highly significant correlation.
- A p-value ≤ 0.05 (*) suggests a significant correlation.

Conclusions and Recommendations

Conclusions

This study concluded the followings:

1. There are higher levels of IL-17A, Copeptin, and hs-CRP in patients with risk factors of IHD than those without, which may indicated the role of these factors in the pathogenesis of IHD.
2. Patients with stable angina, there was a increased significant in the levels of IL-17A & Copeptin, whilst a no increased significant in the concentrations of hs-CRP, FoxP3, & VWF.
3. Patients with unstable angina, there was a increased significant the level of IL-17A, Copeptin, hs-CRP & FoxP3, whilst a no increased significant in the concentrations of VWF.
4. Patients with myocardial infraction, there was a high increased in the levels of IL-17A, Copeptin, hs-CR, FoxP3, VWF, & Troponin I.
5. There were decreased in concentrations of IL-35 in patients with I.H.D. (stable angina, unstable angina and myocardial infraction).

Recommendations

The followed points were recommended by this study:

1. Encouraging the use of IL-17A,and IL-35 biomarkers as early prediction of I.H.D.
2. Conducting studies of immuno-biochemical assessments on the treatments used and their effects of I.H.D.
3. Conducting further studies to clarify the presence of high level of von-Willebrand factor in patients with myocardial infraction although aspirin therapy is used.

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Appendices

Appendix 1 Questionnaire

Name: Age: Sex:

Phone: Sample date:

Clinical problems:

With I.H.D. ☐ Without I.H.D. ☐

Hypertension Diabetes mellitus

BMI Smoking

PR BP

Other diseases:

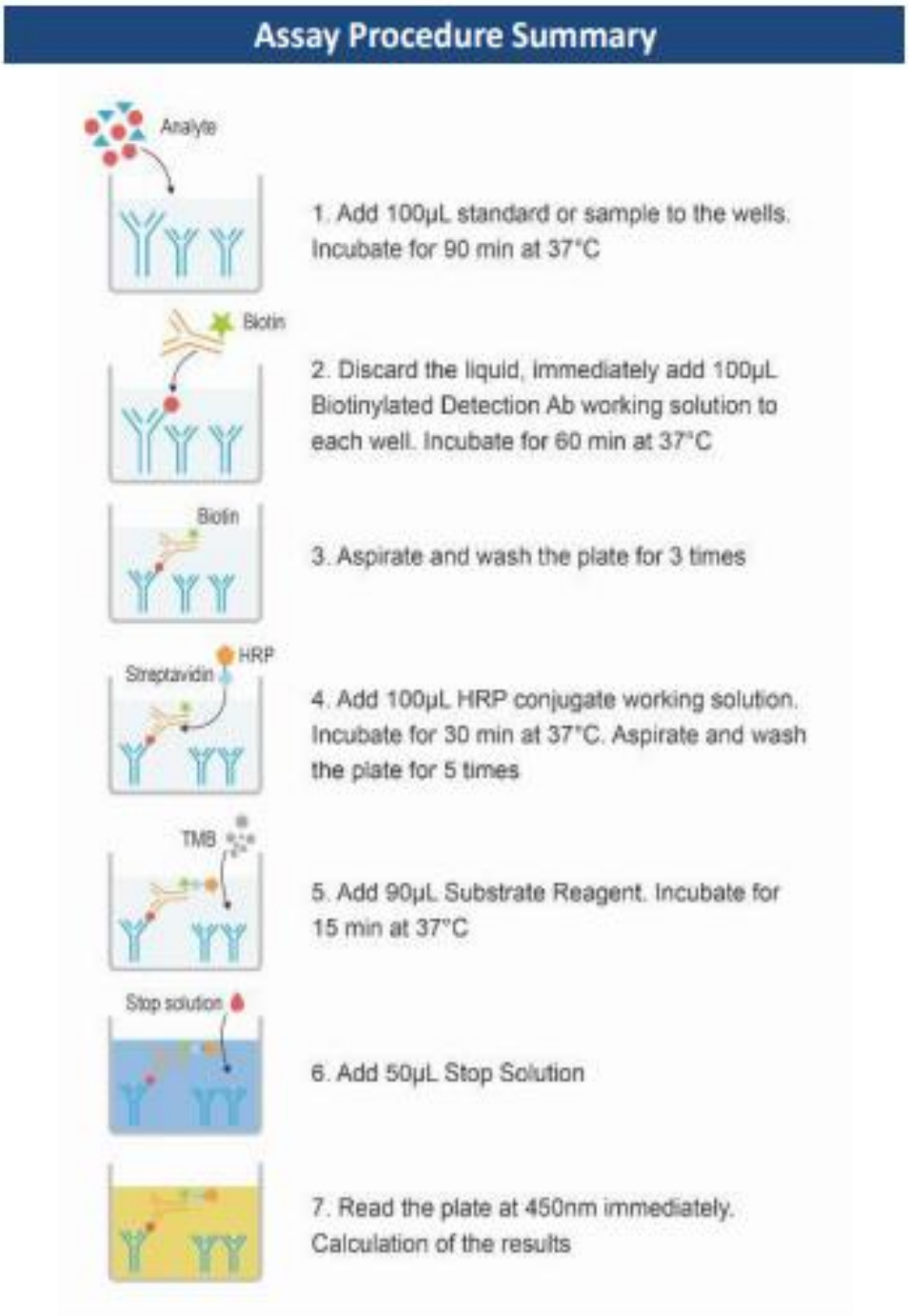
Treatment:

Lab. Investigations:

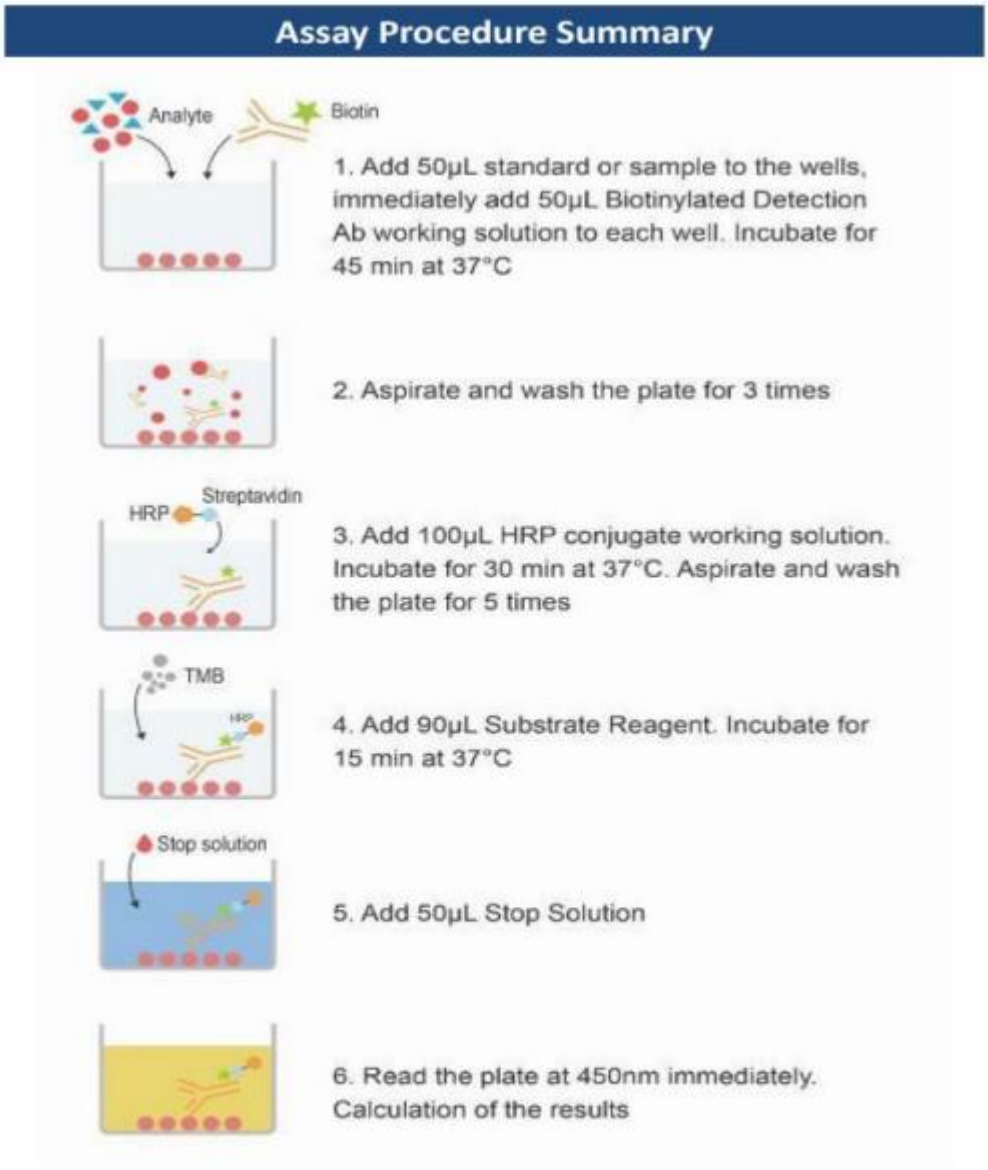
High sensitivity CRP	
Troponin	
Copeptin	
Von Willebrand factor	
TREG. FOXP3	
IL 35	
IL 17	

Appendix 2 Assay procedure

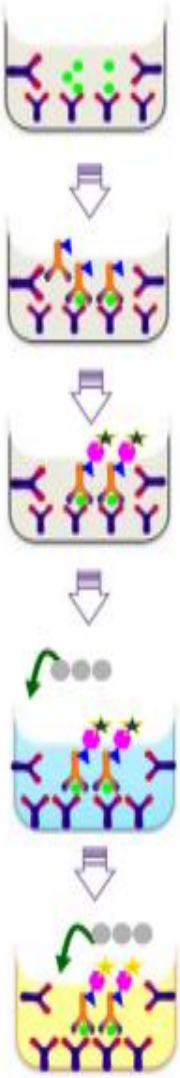
- Troponin I



- Copeptin


Assay Procedure Summary
Analyte
Biotin
1. Add 50μL standard or sample to the wells, immediately add 50μL Biotinylated Detection Ab working solution to each well. Incubate for 45 min at 37°C
2. Aspirate and wash the plate for 3 times
HRP
Streptavidin
3. Add 100μL HRP conjugate working solution. Incubate for 30 min at 37°C. Aspirate and wash the plate for 5 times
TMB
4. Add 90μL Substrate Reagent. Incubate for 15 min at 37°C
Stop solution
5. Add 50μL Stop Solution
6. Read the plate at 450nm immediately. Calculation of the results

- Von Willbrand Factor(VWF)



1. After the kit is equilibrated at room temperature, add 100 µL of Standard Working Buffer (gradually diluted according to the instructions) or 100 µL of sample to each well, and incubate at 37°C for 80 minutes.

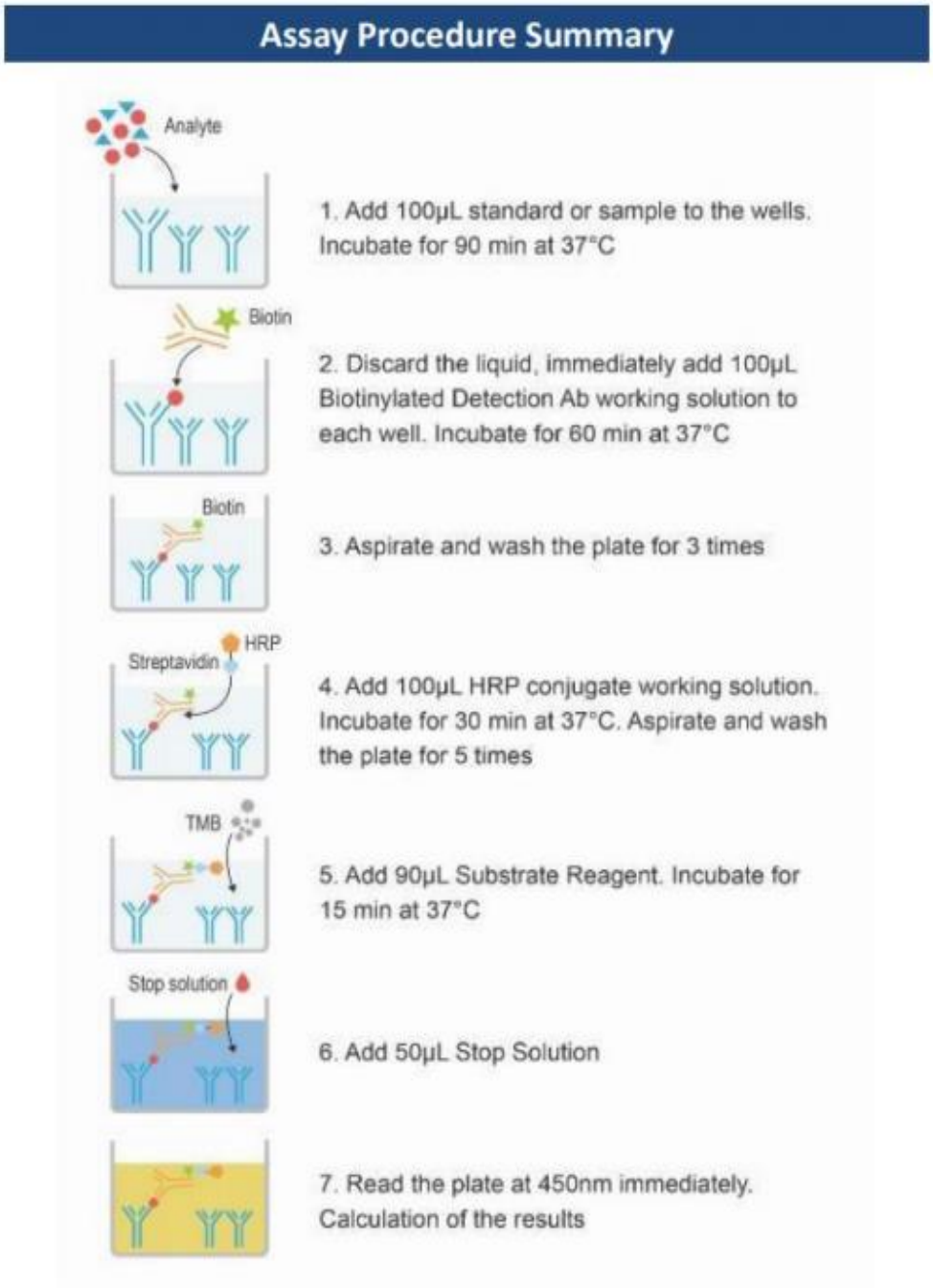
2. Discard the liquid in the plate, add 200 µL 1× Wash Buffer to each well, and wash the plate 3 times. After pat it dry against clean absorbent paper, add 100 µL Biotinylated Antibody Working Solution (1×) to each well, incubate at 37°C for 50 minutes.

3. Discard the liquid in the plate, add 200 µL 1× Wash Buffer to each well, and wash the plate 3 times. After pat it dry against clean absorbent paper, add 100 µL 1× Streptavidin-HRP Working Solution to each well, incubate at 37°C for 50 minutes.

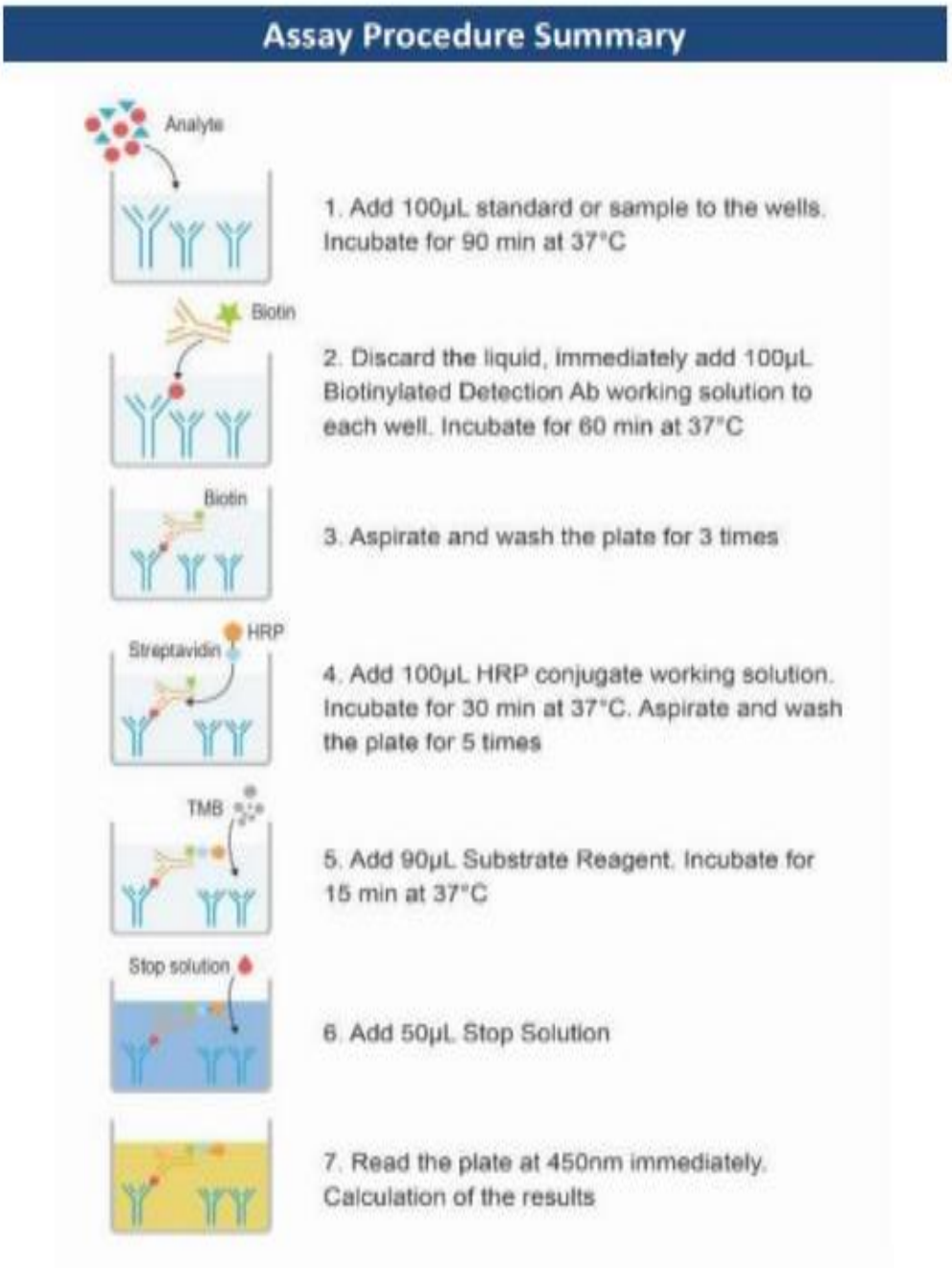
4. Discard the liquid in the plate, add 200 µL 1× Wash Buffer to each well, and wash the plate 5 times. After pat it dry against clean absorbent paper, add 90 µL TMB Substrate Solution to each well, incubate at 37°C for 20 minutes in the dark.

5. Add 50 µL Stop Solution to each well, shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm immediately, calculation of the results.

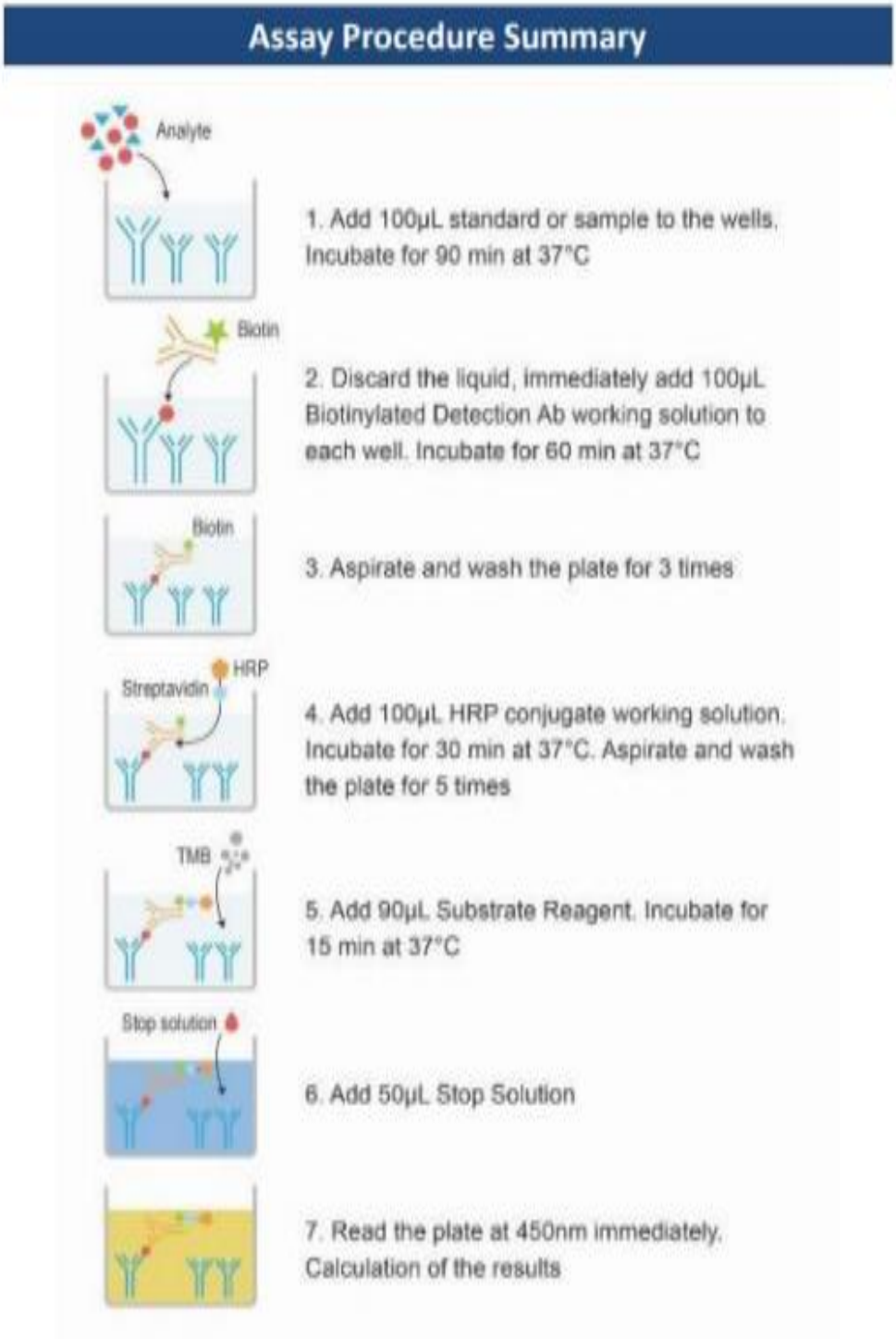
- Forkhead Box Protein P3 (FOXP3)



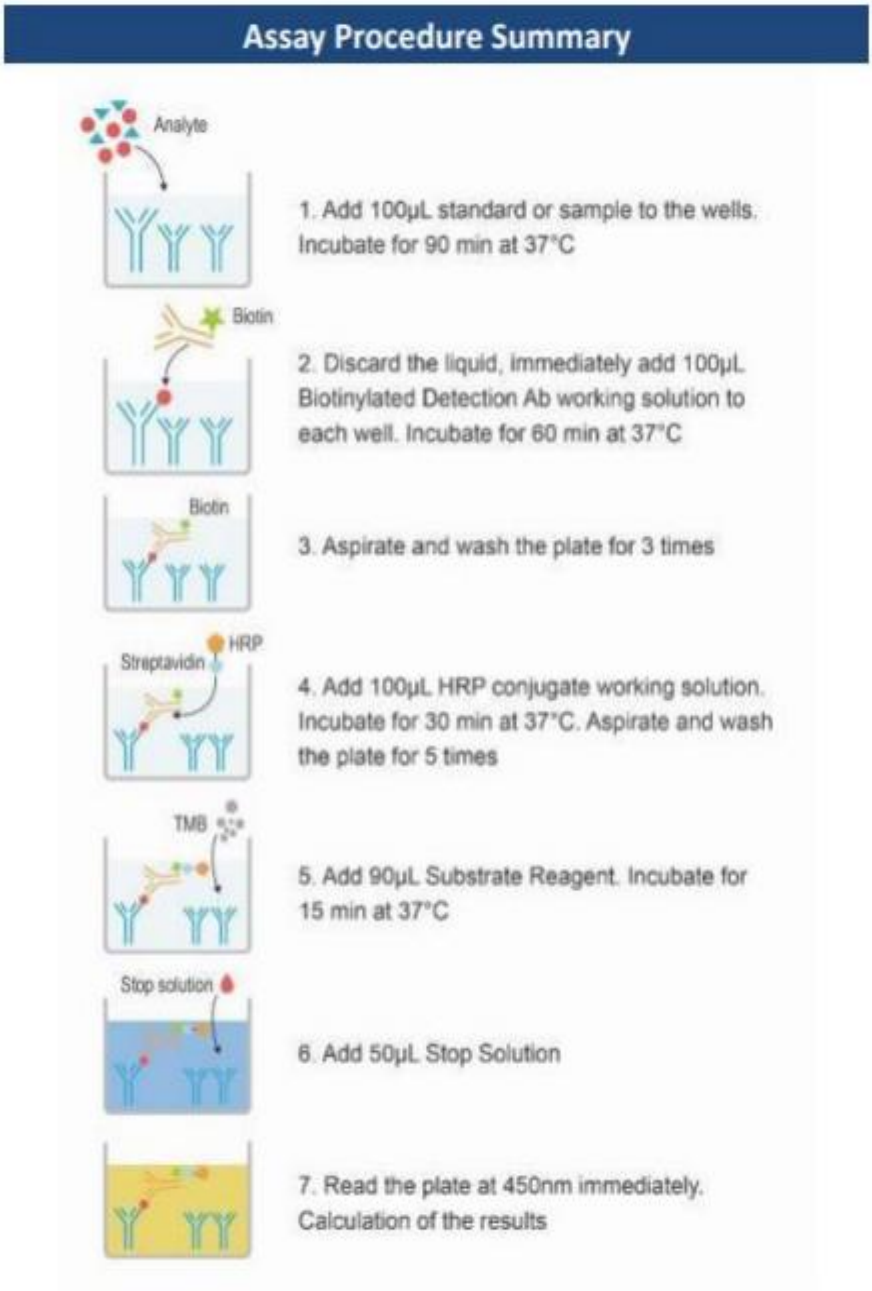
- Interleukin 35 (IL-35)



- Interleukin 17A (IL-17A)



- High-sensitivity C-Reactive Protein(hs-CRP)



Appendix 3

ELISA Reader

Kits of Parameters

Appendix 4 kits

(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSTICS !)

Catalog No: E-EL-H0649

Product size: 96T/48T/24T/96T*5

Elabscience® Human TNNI3/cTn-I(Troponin I Type 3, Cardiac) ELISA Kit

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help (info in the header of each page).

Tel: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please refer to specific expiry date from label outside of box.

Please kindly provide us with the lot number (on the outside of the box) of the kit for more efficient service.

（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSTICS !）

Catalog No: E-EL-H0851

Product size: 96T/48T/24T/96T*5

Elabscience® Human CPP(Copeptin) ELISA Kit

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help (info in the header of each page).

Tel:	1-832-243-6086
Fax:	1-832-243-6017
Email:	techsupport@elabscience.com
Website:	www.elabscience.com

Please refer to specific expiry date from label outside of box.

Please kindly provide us with the lot number (on the outside of the box) of the kit for more efficient service.

ELK Biotechnology

Human vWF(Von Willebrand Factor) ELISA Kit

Cat: ELK5571

For research use only. Not intended for diagnostic use.

Sensitivity: 0.056 ng/mL

Detection Range: 0.16-10 ng/mL

Specificity: This assay has high sensitivity and excellent specificity for detection of Human vWF. No significant cross-reactivity or interference between Human vWF and analogues was observed.

Please refer to the outer packaging label of the kit for the specific shelf life.

KIT Components

Reagents	Quantity		Storage Condition
	48T	96T	
Pre-Coated Microplate	6 strips x 8 wells	12 strips x 8 wells	4°C/-20°C
Standard (Lyophilized)	1 vial	2 vials	4°C/-20°C
Biotinylated Antibody (100×)	60 µL	120 µL	4°C/-20°C
Streptavidin-HRP (100×)	60 µL	120 µL	4°C/-20°C
Standard/Sample Diluent Buffer	10 mL	20 mL	4°C/-20°C
Biotinylated Antibody Diluent	6 mL	12 mL	4°C/-20°C
HRP Diluent	6 mL	12 mL	4°C/-20°C
Wash Buffer (25×)	10 mL	20 mL	4°C/-20°C
TMB Substrate Solution	6 mL	10 mL	4°C/-20°C (store in dark)
Stop Reagent	3 mL	6 mL	4°C/-20°C
Plate Covers	1 piece	2 pieces	RT

(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSTICS !)

Catalog No: E-EL-H5134

Product size: 96T/48T/24T/96T*5

Elabscience® Human hs-CRP(high-sensitivity C-Reactive Protein) ELISA Kit

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help (info in the header of each page).

Tel: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please refer to specific expiry date from label outside of box.

Please kindly provide us with the lot number (on the outside of the box) of the kit for more efficient service.

（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSTICS !）

Catalog No: E-EL-H1104

Product size: 96T/48T/24T/96T*5

Elabscience® Human FOXP3(Forkhead Box Protein P3) ELISA Kit

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help (info in the header of each page).

Tel: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please refer to specific expiry date from label outside of box.

Please kindly provide us with the lot number (on the outside of the box) of the kit for more efficient service.

(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSTICS !)

Catalog No: E-EL-H0105

Product size: 96T/48T/24T/96T*5

Elabscience® Human IL-17A(Interleukin 17A) ELISA Kit

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help (info in the header of each page).

Tel: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please refer to specific expiry date from label outside of box.

Please kindly provide us with the lot number (on the outside of the box) of the kit for more efficient service.

(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSTICS !)

Catalog No: E-EL-H2443

Product size: 96T/48T/24T/96T*5

Elabscience® Human IL-35(Interleukin 35) ELISA Kit

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help (info in the header of each page).

Tel: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please refer to specific expiry date from label outside of box.

Please kindly provide us with the lot number (on the outside of the box) of the kit for more efficient service.

Appendix 5 Tables of Data

Table 1: The Concentration of high-sensitivity C-reactive protein (hs-CRP) in patients with and without Ischemic heart diseases .

Level	Mean	Std. Deviation	P. value	Duncan Post hoc Test
Control	117.21	7.50	0.0008	a
With Risk Factor	249.77	74.30		b
Stable Angina	200.00	53.12		b
Unstable Angina	417.75	127.16		c
Myocardial Infraction	782.60	143.68		d

Table 2: The Concentration of Troponin I in patients with and without Ischemic heart diseases.

Level	Mean	Std. Deviation	P. value	Duncan Post hoc Test
Control	0.022	0.002	0.0001	a
With Risk Factor	0.019	0.004		a
Stable Angina	0.041	0.011		b
Unstable Angina	0.039	0.005		b
Myocardial Infraction	0.066	0.013		c

Table 3: The Concentration of Copeptin in patients with and without Ischemic heart diseases.

Level	Mean	Std. Deviation	P. value	Duncan Post hoc Test
Control	253.51	10.88	0.0003	a
With Risk Factor	394.57	101.96		b
Stable Angina	425.18	105.53		b
Unstable Angina	517.57	126.84		c
Myocardial Infraction	907.75	125.57		d

Table 4: The Concentration of Von Willbrand Factors in patients with and without Ischemic heart diseases.

Level	Mean	Std. Deviation	P. value	Duncan Post hoc Test
Control	0.24	0.16	0.0005	a
With Risk Factor	0.94	0.80		b
Stable Angina	1.44	0.65		c
Unstable Angina	2.92	0.35		d
Myocardial Infraction	4.57	0.99		e

Table 5: The Concentration of FOXP3 in patients with and without Ischemic heart diseases .

Level	Mean	Std. Deviation	P. value	Duncan Post hoc Test
Control	1.48	0.32	0.0001	a
With Risk Factor	2.83	1.03		b
Stable Angina	3.68	1.50		c
Unstable Angina	5.51	1.04		d
Myocardial Infraction	10.64	1.15		e

Table 6: The Concentration of interlukin-35(IL-35) in patients with and without Ischemic heart diseases .

Level	Mean	Std. Deviation	P. value	Duncan Post hoc Test
Control	405.90	112.46	0.0002	b
With Risk Factor	157.87	66.67		a
Stable Angina	160.96	52.51		a
Unstable Angina	160.35	43.41		a
Myocardial Infraction	146.15	40.16		a

Table 7: The Concentration of interlukin-17A(IL-17A) in patients with and without Ischemic heart diseases.

Level	Mean	Std. Deviation	P. value	Duncan Post hoc Test
Control	91.40	37.56	0.0004	a
With Risk Factor	333.70	87.24		b
Stable Angina	258.56	132.78		b
Unstable Angina	491.64	202.69		c
Myocardial Infraction	944.38	172.02		d

Table 8: The Concentration of HbA1c In Patients With And Without Ischemic Heart Diseases.

Level	Mean	Std. Deviation	P. value	Duncan Post hoc Test
Control	4.62	0.44	0.0002	a
With Risk Factor	7.16	2.49		b
Stable Angina	7.83	2.52		b
Unstable Angina	7.31	1.69		b
Myocardial Infraction	8.44	1.92		b

الخلاصة

يعد مرض القلب الإقفاري (IHD) مرض يصيب القلب ويحدث نتيجة لنقص توزيع الأوكسجين على عضلة القلب، ويحدث بشكل رئيسي بسبب انسداد الشرايين نتيجة لتراكم الكوليسترول على الجدران. يستخدم الإقفار لتعريف "انخفاض إمداد الدم". تزود الشرايين التاجية عضلة القلب بالدم، وقد يبدأ انسداد الشريان التاجي في تقليل إمداد الدم إلى القلب.

وتكونت الدراسة من (100) شخص مقسمين الى مجموعتين رئيسيتين (مجموعة الاصحاء ومجموعة المرضى من كلا الجنسين ذكور واناث بعمر (80-25) تم جمع عينات الدم في الفترة من اذار الى اغسطس 2024 في مستشفى الامام الحسن المجتبى التعليمي ومركز كربلاء للأمراض وجراحة القلب في مستشفى الامام الحسين التعليمي في محافظة كربلاء.

تمت هذه الدراسة من خلال مراقبة بعض المعايير المناعية والكيميائية الحيوية مثل IL_35 و IL-17A و copeptin و VWF و FoxP3 و hs-CRP في المرضى وتم تحديد هذه المعايير باستخدام اختبار تقنية الاقتران المناعي المرتبط بالأنزيم (ELISA).

ازداد تركيز Troponin I بشكل كبير ($P < 0.00001$) في احتشاء عضلة القلب (0.066 ng/ml) مقارنة بالذبحة الصدرية المستقرة ، الذبحة الصدرية غير المستقرة (0.039 ng/ml ,0.041) على التوالي ولا يوجد زيادة كبيرة بين الذبحة الصدرية المستقرة وغير المستقرة.

ازداد تركيز Copeptin تدريجيًا بشكل كبير ($P < 0.00001$) في احتشاء عضلة القلب (907.75pg/mL) مقارنة بالمعايير الأخرى الاصحاء ,مع عوامل الخطر، الذبحة الصدرية المستقرة وغير المستقرة (517.57pg/ml , 425.18, 344.57, 253.51) على التوالي.

تزداد الذبحة الصدرية غير المستقرة (517.57 pg/ml) بشكل كبير ($P < 0.05$) مقارنة بالذبحة الصدرية المستقرة (425.18 pg/ml).

ازداد تركيز VWF يتزايد تدريجيا بشكل ملحوظ ($P<0.00001$) في احتشاء عضلة القلب (4.57 ng/ml) مقارنة بالمعايير الأخرى (الاصحاء, عوامل الخطر، الذبحة الصدرية المستقرة، والذبحة الصدرية غير المستقرة (2.92 ng/ml , 1.44, 0.94, 0.24) على التوالي وأيضا ازدات الذبحة الصدرية غير المستقرة (2.92 ng/ml) بشكل ملحوظ ($P<0.00001$) مقارنة بالذبحة الصدرية المستقرة (1.44 ng/ml).

ازداد تركيز hs-CRP بشكل ملحوظ فجأة ($P<0.00001$) في الذبحة الصدرية غير المستقرة ويزداد بشكل مستمر في احتشاء عضلة القلب (782.60 pg/ml, 417.75) على التوالي مقارنة بالمعايير الأخرى (الاصحاء, ومع عوامل الخطر، الذبحة الصدرية المستقرة

(200.00 pg/ml, 249.77, 117.21)على التوالي، اشارت هذه النتيجة إلى أن بروتين سي التفاعلي عالي الحساسية هو مؤشر لحدوث احتشاء عضلة القلب.

ازداد تركيز Foxp3 بشكل مفاجئ ($p<0.00001$) في الذبحة الصدرية غير المستقرة وازداد بشكل مستمر بشكل كبير في احتشاء عضلة القلب (10.64 ng/ml, 5.51)على التوالي مقارنة

بالمعايير الأخرى (الاصحاء ,مع عوامل الخطر، الذبحة الصدرية المستقرة)
(1.48, 2.83, 3.68 ng/ml)على التوالي، اشارة هذه النتيجة إلى أن Foxp3 هو مؤشر لحدوث احتشاء عضلة القلب.

ازاد تركيز IL-17A بشكل كبير (P <0.00001) في الذبحة الصدرية غير المستقرة وازداد بشكل كبير باستمرار في احتشاء عضلة القلب (491.64, 944.38pg/ml) على التوالي مقارنة بالمعايير الأخرى (الاصحاء ,مع عوامل الخطر، الذبحة الصدرية المستقرة
(91.40, 333.70, 258.56 pg/ml)على التوالي. اشارة هذه النتيجة إلى IL17 كمؤشر على احتشاء عضلة القلب.

انخفض تركيز IL-35 بشكل كبير (P <0.00001) في احتشاء عضلة القلب
(146.15 pg/ml) مقارنة بالمجموعة الاصحاء (405.90pg/ml) وكل هذا انخفاض كبير مع المعايير الأخرى (مع عامل الخطر، الذبحة الصدرية المستقرة، الذبحة الصدرية غير المستقرة (157.87, 160.96, 160.35pg/ml)على التوالي.

أظهرت الدراسة زيادة في تركيزات hs-CRP وIL-17A وFoxP3، بالإضافة إلى انخفاض في مستويات IL-35، لدى المرضى المصابين بالذبحة الصدرية المستقرة، والذبحة الصدرية غير المستقرة، واحتشاء العضلة القلبية. علاوة على ذلك، كانت تركيزات Troponin I أعلى بشكل ملحوظ في حالات احتشاء العضلة القلبية مقارنة بالحالات الأخرى (بما في ذلك الأفراد الأصحاء، والأشخاص ذوي العوامل المسببة، والذبحة الصدرية المستقرة وغير المستقرة)، مما يبرز دورها كعلامة حيوية رئيسية للإصابة القلبية. كما ارتفعت مستويات Copeptin بشكل كبير في الذبحة الصدرية غير المستقرة، واستمرت في الارتفاع بشكل ملحوظ في احتشاء العضلة القلبية. وتشير الدراسة أيضًا إلى أن VWF قد يكون علامة حيوية تنبؤية، حيث ازادت مستوياته بشكل كبير في الذبحة الصدرية غير المستقرة، واستمرت في الارتفاع بشكل ملحوظ في احتشاء العضلة القلبية.

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رسالة مقدمة

الى مجلس كلية العلوم الطبية الطبيقية - جامعة كربلاء

وهي جزء من متطلبات نيل شهادة الماجستير في التحليلات المرضية

كتبت بواسطة

داليا جاسم محمد

بكالوريوس تحليلات مرضية / كلية العلوم الطبية التطبيقية / جامعة كربلاء,2021

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