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Association of Soluble Suppression of Tumorigenicity 2 (sST2) and Insulin-like Growth Factor Binding Protein 2 (IGFBP-2) with Cardiac Risk Score in Iraqi Patients with Unstable Angina \ Non-ST-Elevation Myocardial Infarction

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

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بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

﴿ وَأَن لَّيْسَ لِلْإِنسَٰنِ إِلَّا مَا سَعَىٰ (39) وَأَنَّ سَعْيَهُۥ سَوْفَ يُرَىٰ (40) ثُمَّ يُجْزَىٰهُ ٱلْجَزَآءَ ٱلْأَوْفَىٰ (41)﴾

صدق الله العلي العظيم

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Supervisor Certification

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Association of Soluble suppression of tumorigenicity 2 (sST2) and Insulin-like Growth Factor Binding Protein 2 (IGFBP-2) with Cardiac Risk Score in Iraqi patients with Unstable Angina \ Non-ST-Elevation Myocardial Infarction

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

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We, the examining committee certify that we have read this M.Sc. thesis entitled: **(Association of Soluble Suppression of Tumorigenicity 2 (sST2) and Insulin-like Growth Factor Binding Protein 2 (IGFBP-2) with Cardiac Risk Score in Iraqi Patients with Unstable Angina \ Non-ST-Elevation Myocardial Infarction)**

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Dedication

With love, I dedicate this work to the soul of my martyred grandfather

And to my biggest supporter (my father), and the one who alleviated my difficulties with her prayers (my mother) and all my family and friends who have supported me along the way

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First, and above all, thanks to the Great Merciful Almighty (Allah) Who gave me health, strength, patience, and perseverance and facilitated the ways for me to accomplish this work.

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Summary

Both Non-ST-Elevation Myocardial Infarction (NSTEMI) and Unstable angina (UA) fall under the category of non-ST elevation acute coronary syndrome (NSTE-ACS). However, NSTEMI is differentiated from UA by the presence of acute myocardial necrosis. conducted an immediate risk assessment is crucial in order to determine whether persons with UA/NSTEMI require early invasive intervention. The Reynolds score, heart score, and TIMI score are prognostic tools used to evaluate the risk of cardiovascular events based on several variables. Suppression of Tumorigenicity 2(ST2), belonging to the Interleukin-1(IL-1) receptor family, exists in two forms: Suppression of Tumorigenicity 2 ligand(ST2L), which is a receptor linked to the cell membrane, and Soluble Suppression of Tumorigenicity 2(sST2), which is a soluble version found in the bloodstream. Insulin-like Growth Factor Binding Protein 2(IGFBP2), a constituent of the IGFBP family, modulates the activity of Insulin-like growth factors (IGFs) by engaging with them in the circulatory system and impeding their attachment to IGF receptors.

This study aimed to examine the correlation between sST2 and IGFBP-2 levels with TIMI score, Reynolds score, and heart score in patients with NSTE-ACS. The objective was to categorize the risk into low, moderate, and high based on clinical criteria. This categorization can assist clinicians in making more educated decisions regarding patient treatment options.

The present study is a case-control study includes 90 individuals aged between 44-70 years. (30 patients with NSTEMI, 30 patients with UA, and 30 individuals as a control group). The study was conducted at the Kerbala Heart Center. Where Specialist physicians assessed and diagnosed the patients. The participants were gathered over the duration spanning from October 2023 to January 2024 A questionnaire was conducted to gather patient information, including demographics, risk factors, medical history, physical examination results, and a

range of blood tests such as sST2, IGFBP2, Pro_BNP, Hs-Troponin I, lipid profile test, hs-CRP, and creatinine levels.

Patients with NSTE-ACS had a significant decrease in The concentration of sST2 ($p \leq 0.000$) compared to the control group, there was no statistically significant difference in the level of IGFBP2 ($p=0.560$) between the patient group and the control group. On the other hand, There were no significant variations observed in the levels of sST2 and IGFBP2 among patients who were classified into three risk groups: low-risk, Medium-risk, and high-risk. The classification was based on the TIMI score, Reynolds score, and Heart score. The results of the receiver operating curve (ROC) demonstrated that sST2 had a fair performance as a diagnostic parameter for patients with NSTEMI and UA when compared to the control group. Additionally, the IGFBP2 level exhibited a fair performance as a diagnostic parameter for patients with UA when compared to the NSTEMI patients

In Conclusion patients with NSTE-ACS, exhibited a lower level of sST2 compared to control group possibly due to B-Blocker and ACE inhibitor medications enhancing left ventricle function and IL-33/ST2 signaling. On the other hand, there was no significant differences in IGFBP2 levels between the patient and control groups. However, the study found no significant differences in sST2 and IGFBP2 levels across different risk categories based on TIMI Score, Reynolds Score, and Heart Score in patient with NSTE-ACS. As a result, it was not possible to classify patients into Low, moderate, and high-risk groups using these biomarkers.

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List of Abbreviations

CAD	Coronary artery disease
ACEIs	angiotensin-converting enzyme inhibitors
ACS	Acute Coronary Syndrome
AMI	Acute Myocardial Infarction
AS	Atherosclerosis
ASCVD	Atherosclerotic Cardiovascular Disease
AUC	Area under curve
B- blockers	beta-blockers
BMI	body mass index
BP	Blood pressure
CCTA	Coronary computed tomography angiography
CHD	Coronary heart disease
CI	Confidence Interval
CMR	Cardiac magnetic resonance
CRP	C-reactive protein
CS	Cardiogenic Shock

cTn	cardiac Troponin
CVDs	Cardiovascular diseases
ECG	electrocardiogram
ECM	Extracellular matrix
Echo.	Echocardiography
EH	essential hypertension
ELISA	Enzyme-Linked Immunosorbent Assay
EPC	endothelial progenitor cells
FH Premature CAD	family history of premature coronary artery disease
HDL	High-density Lipoprotein
HF	Heart failure
Hs-CRP	High sensitivity-C reactive protein
hs-cTn	high-sensitivity troponins
HTN	Hypertension
ICA	Invasive coronary angiography
IGF	insulin-like growth factor
IGF-1	Insulin-like Growth Factor -1
IGF-1R	Insulin-like growth factor receptors I
IGF-2R	Insulin-like growth factor receptors II
IGFBP-2	Insulin-like Growth Factor Binding Protein 2
IGFBPs	Insulin-like Growth Factor -binding proteins
IL-33	interleukin-33
IQR	Interquartile Range
LDL	Low-density Lipoprotein
LV	left ventricular
MACE	major adverse cardiac events
MD	Microvascular dysfunction
MI	Myocardial infarction

MMP	Matrix metalloproteinase
NSTE-ACS	non-ST elevation acute coronary syndrome
NSTEMI	non-ST elevation myocardial infarction
OCT	Optical coherence tomography
OR	Odds Ratio
PE	pulmonary embolism
Prp-BNP	pro-brain natriuretic peptide
r	pearson correlation coefficients
RAAS	Renin-angiotensin-aldosterone system
ROC	receiver operating characteristics
RRS	Reynolds risk
RV	Right ventricular
SCAD	Spontaneous coronary artery dissection
SPR	Solid Phase Receptacle
sST2	Soluble Suppression of Tumorigenicity 2
ST2	Suppression of Tumorigenicity 2
ST2L	Suppression of Tumorigenicity 2 ligand
STEMI	ST-elevation myocardial infarction
T2DM	Type 2 Diabetes mellitus
TG	Triglycerides
TIMI	thrombolysis in myocardial infarction risk index
TTE	Transthoracic echocardiogram
UA/NSTEMI	Unstable angina/non-ST elevation myocardial infarction
UDMI	Universal Definition of Myocardial Infarction
VSMCs	Vascular smooth muscle cells
WHO	World Health Organisation

Chapter One

Introduction and Literature Review

1. Introduction

1.1. coronary artery disease

The coronary arteries are responsible for delivering blood to the cardiac muscle and providing it with the essential components required for its proper functioning. The term "Coronary artery disease" (CAD) refers to the constriction of these arteries, resulting from the buildup of atherosclerotic material within their lumen. Myocardial ischemia occurs when the heart muscle does not receive enough blood due to stenosis, particularly during times of elevated demand (Pagliaro et al., 2020).

Moreover, the cause of CAD is a very intricate process, with several interconnected elements contributing to the development of the disease. The clinical manifestations of CAD include arrhythmia, angina, myocardial infarction, and heart failure. (Ullah et al., 2023). The narrowing of the lumen of the epicardial coronary arteries and the restriction of blood flow through them is caused by the growth of atherosclerotic plaques on their walls, lifestyle modifications and medication intervention can effectively postpone or potentially prevent the onset of CAD (Trigka and Dritsas, 2023).

Stage of coronary artery disease include (Fox et al., 2020) :

- The asymptomatic period: refers to the phase of atherosclerosis during which no symptoms are present, in addition, individuals without significant narrowing of the coronary arteries may not experience any symptoms, even if they have atherosclerotic lesions in those arteries (Trigka and Dritsas, 2023).
- Stable angina: Angina pain can manifest either during physical exertion or during periods of heightened emotional stress, stable angina is often a reasonably benign medical condition that allows for the identification and implementation of suitable treatment options. (Reeh et al., 2019).

- Unstable angina: The occurrence of angina pain during periods of inactivity. This variant of coronary artery disease is considered more perilous, so it is referred to as pre-infarction angina, it is evident that hospitalisation is necessary to treat this unstable situation promptly and administer adequate medication to prevent the unwanted progression to myocardial infarction (Goyal and Zeltser, 2022).
- Acute myocardial infarction: It is characterized by the death of a portion of the heart muscle. It presents with angina that is persistent, does not alleviate with rest, and lasts for more than thirty minutes, the immediate transfer of the patient to a hospital is imperative (Shao et al., 2020).
- Sudden cardiac arrest: This is the most severe presentation of the complete range of clinical symptoms associated with coronary artery disease (Trigka and Dritsas, 2023)

Coronary artery disease CAD is classified into two main categories: (Knuuti et al., 2020)

- Chronic coronary syndromes (CCS)
- Acute coronary syndromes (ACS).

The categorization of acute coronary syndrome is typically done as below: (Shahjehan and Bhutta, 2020)

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

1.2. Acute coronary syndrome (ACS)

The term "acute coronary syndromes" (ACS) refers to a variety of illnesses, which include individuals presenting with recent changes in clinical symptoms or signs , with or without alterations in electrocardiogram (ECG), as well as with or without sudden elevations in cardiac troponin (cTn) concentrations (Byrne et al., 2024). STEMI, NSTEMI, and UA are all included in the phrase "acute coronary

syndrome" (ACS) (Pollack et al., 2020). ACS is characterized by sudden onset substernal chest pain or pressure that frequently extends to the left arm and neck. It may also include other symptoms such as dyspnea, palpitations, confusion, syncope, cardiac arrest, or newly developed congestive heart failure (Shahjehan and Bhutta, 2020).

The utilization of the term acute myocardial infarction (AMI) is appropriate in cases where there is observable evidence of myocardial injury, as indicated by an elevation in cardiac troponin levels, with at least one value surpassing the upper reference limit of the 99th percentile. (Ibanez et al., 2018). AMI can be categorised into two main types: ST-elevation myocardial infarction (STEMI) and non-ST elevation myocardial infarction (NSTEMI).Based on the electrocardiogram (ECG) obtained upon the patient's admission, and this classification aids in guiding therapeutic interventions during the acute phase (Martínez et al., 2022).

Unstable angina, which has clinical signs of ACS but no biochemical evidence of myocardial infarction, is at the milder end of the range of acute coronary syndrome (Bergmark et al., 2022).

The 4th Universal Definition of Myocardial Infarction (UDMI) states that in order to classify atherothrombotic coronary artery disease as either NSTEMI or STEMI, it is necessary to observe a rise or fall in cardiac troponin (cTn) level (or another biomarker if cTn is not available). This classification is based on ECG findings, along with signs of clinical ischaemia (such as symptoms, changes in the electrocardiogram, further imaging findings, or signs of coronary thrombus). (Thygesen et al., 2018).

Acute myocardial infarction (AMI) is the primary aetiology of cardiogenic shock (CS) and is correlated with increased mortality rates, despite the application of contemporary intensive treatments and coronary revascularization procedures (García-García et al., 2020, Harjola et al., 2015, Martínez et al., 2022).

Comprehensive investigations have been conducted to examine the mortality rates among patients diagnosed with STEMI and NSTEMI . The findings of these investigations have indicated that STEMI patients exhibit a greater incidence of in-hospital mortality, whereas NSTEMI patients experience a relatively poorer long-term prognosis (García-García et al., 2011, Polonski et al., 2011, Martínez et al., 2022) .

1.2.1. Unstable angina \ Non-ST-Elevation Myocardial Infarction (NSTE-ACS)

Non-ST elevation myocardial infarction (NSTEMI) and unstable angina (UA) are both classified as non-ST elevation acute coronary syndrome (NSTEACS). These conditions exhibit similarities in terms of aetiology and prognosis. However, NSTEMI is distinguished from UA by the occurrence of acute myocardial necrosis (Puelacher et al., 2019). NSTE-ACS accounts for approximately 75% of all cases of acute coronary syndrome (ACS), and its occurrence has steadily risen over the last ten years (Zhao et al., 2023). Cardiac biomarkers are employed to distinguish between NSTEMI , characterised by positive biomarker results, and UA, characterised by negative biomarker results, in cases of acute coronary syndrome when there are no ST elevations observed on the electrocardiogram (ECG) (Evanchan, 2020). Twenty-five years ago, approximately 50% of individuals with NSTEACS were diagnosed with unstable angina. and due to the widespread use of sensitive troponin assays, unstable angina is expected to be marginalized. However, it still contributes significantly to the occurrence of acute coronary syndromes (Piatek et al., 2020). Over the past decade, there have been notable advancements in the diagnosis and treatment of UA, including the utilization of more sensitive troponin tests and the emergence of coronary CT angiography (CCTA) as a viable alternative to invasive coronary angiography (ICA) (Collet et al., 2021). Although UA and NSTEMI have a common pathophysiology, their distinguishing factor is in the extent of ischemia-induced cardiac damage, which determines the release of detectable levels of

myocardial injury markers (Piatek et al., 2020).The introduction of high-sensitivity troponins (hs-cTn) resulted in a 20% relative increase in the identification of NSTEMI, accompanied by a corresponding reduction in the diagnosis of UA (Fladseth et al., 2022). Both UA and NSTEMI present with symptoms and indications of myocardial ischemia during periods of rest or minimal physical activity (Puelacher et al., 2019). It is recommended that individuals diagnosed with UA/NSTEMI undergo immediately risk stratification in order to determine the necessity of an early invasive intervention (Rahmani et al., 2020).

1.2.1.1 Prevalence

The worldwide incidence of cardiovascular diseases (CVDs) experienced a twofold increase, rising from 271 million cases in 1990 to 523 million cases in 2019. Additionally, the mortality rate associated with CVDs reached 18.6 million cases globally (Roth et al., 2020) Annually, a global estimate indicates that over 7 million individuals receive a diagnosis of Acute Coronary Syndrome (ACS) (Bhatt et al., 2022). In the United States of America, an estimated 1.36 million hospitalisations are necessitated annually due to ACS ,different Middle Eastern nations had varying rates of ACS prevalence. in Egypt, for example, it was 8.3% in 2001, 13% in Lebanon in2008, and 6% in Saudi Arabia in 2004 (Mirza et al., 2018). However, it is projected that by the year 2030, the prevalence of these conditions will increase as a result of elevated rates of hypertension, diabetes mellitus, overweight and obesity, physical inactivity, smoking, and dyslipidemia (Ahmed et al., 2017). The prevalence of ACS is on the rise globally (Alkhaqani, 2023). NSTEMI account for approximately 2-2.5 million hospital admissions worldwide annually. Patients exhibiting symptoms of high-risk unstable angina are at a heightened risk of mortality (Bhatia, 2023). Myocardial infarction (MI) is the primary clinical presentation of coronary heart disease (CHD) and has presented a substantial burden on both high-income and low-income countries

(Feng et al., 2019). The prevalence of myocardial infarction (MI) has been steadily increasing, making it a significant global public health concern in recent years (Roth et al., 2020, He et al., 2023). The use of therapeutic interventions and lifestyle modifications reduces the occurrence of STEMI, resulting in a higher proportion of NSTEMI cases annually. Currently, around 60% to 75% of AMI cases are classified as NSTEMI (Purnamawaty et al., 2020).

1.2.1.2. Pathogenesis

Acute coronary syndrome (ACS) is defined by a rapid reduction in blood flow to the heart, typically caused by either blood clots in the coronary arteries or other non-atherosclerotic factors. This condition can lead to different types of heart attacks, including STEMI, NSTEMI, and UA (Bergmark et al., 2022). All types of ACS exhibit a shared pathophysiological foundation, characterised by an unstable atherosclerotic plaque that is further worsened by either occlusive or non-occlusive thrombosis (Kheifets et al., 2021, Savovic et al., 2023). ACS is diagnosed in over 7 million people globally annually (Bhatt et al., 2022). Consequently, there has been significant investigation on the pathophysiology of ACS, resulting in the identification of three primary pathways (Yuan et al., 2023).

A. Plaque Rupture

Plaque rupture occurs when the fibrous tissue layer that protects the lipid-rich necrotic core of a plaque breaks or develops cracks (Marchini et al., 2020). Allowing blood to come into contact with substances that encourage blood clot formation, such as tissue factors found in the fatty core. Consequently, a blood clot is formed (Marx et al., 2019). The plaque is often identified by a large lipid core that contains macrophage foam cells and other debris. It is also characterised by a thin fibrous cap, which is less than 65 mm in thickness, and is composed of an abundance of extracellular matrix (ECM). This fibrous cap covers the lipid necrotic core of the plaque (Wirka et al., 2019).

Coronary thrombosis resulting from the rupture of plaque can be categorised as either with or without systemic inflammation, due to the growing focus on direct anti-inflammatory therapies that specifically target atherosclerosis (AS) in recent years, this categorization of plaque rupture may have greater importance (Denegri and Boriani, 2021):

- The initial hypothesis involves the rupture of plaque accompanied by systemic inflammation. Patients with ACS frequently exhibit a significant elevation in C-reactive protein (CRP), indicating the presence of widespread inflammation in ACS (Denegri and Boriani, 2021). Activated macrophages secrete enzymes such as Matrix metalloproteinase (MMP) and cathepsin that break down the components of plaque matrix, hence promoting the degradation of plaque extracellular matrix (Olejarz et al., 2020)
- Plaque rupture without systemic inflammation is another cause of ACS. In cases of plaque rupture without systemic inflammatory activation, additional factors such as severe mood disorders, intensive physical activity, and aberrant mechanical stress on the artery wall may contribute to the occurrence of plaque rupture, psychological stress can result in the rupture of plaque due to the activation of the sympathetic nervous system and the production of catecholamines. This leads to a rise in heart rate, blood pressure, and constriction of the coronary arteries (Yuan et al., 2023).

B. Plaque erosion

Plaque erosion refers to the occurrence of thrombus development mostly in the region of endothelial desquamation next to the atherosclerotic plaque, without causing damage to the fibrous cap that covers the plaque tissue (Yuan et al., 2023). The notion of "vulnerable plaque" has sparked curiosity in plaque erosion as an alternate mechanism of ACS . Recent studies emphasis the

significance of erosion, since it leads to coronary events characterised by intact fibrous caps and elevated extracellular matrix (ECM) molecules, eroded plaques exhibit preserved fibrous capping and elevated levels of ECM molecules. For instance, the levels of proteoglycan and glycosaminoglycan's rise, particularly hyaluronic acid (Kolte et al., 2021). Vascular smooth muscle cells (VSMCs) are present in large quantities in eroded plaques, but leukocytes, specifically macrophages, are less densely clustered, and there is a deficiency of lipids (Chiorescu et al., 2022). The thrombus found in plaque erosion is composed of platelets and is commonly referred to as the "white thrombus," in contrast to the fibrin and red blood cell-rich "red thrombus" observed in plaque rupture (Luo et al., 2021). Plaque erosion exhibits distinct differences from plaque rupture in terms of its epidemiology and clinical manifestations. Plaque erosion is more prevalent in women, younger individuals, and those with a lower occurrence of traditional cardiovascular risk factors (Sato et al., 2022). Plaque erosion patients in ACS have lower plaque burden, less complex lesions, fewer adverse cardiac events (Yamamoto et al., 2019). Patients diagnosed ACS and plaque erosion, as determined by optical coherence tomography (OCT), exhibit reduced levels of inflammatory markers (such as high sensitivity C-reactive protein and white blood cell count) and a more advantageous lipid profile in comparison to those with plaque rupture. Additionally, patients with plaque erosion, as opposed to plaque rupture, demonstrate elevated hemoglobin concentrations (Kolte et al., 2021). Plaque erosion is commonly linked to a presentation of ACS known as NSTEMI, whereas plaque rupture is more frequently observed in individuals with STEMI (As in figure (1-1)) (Baaten et al., 2024).

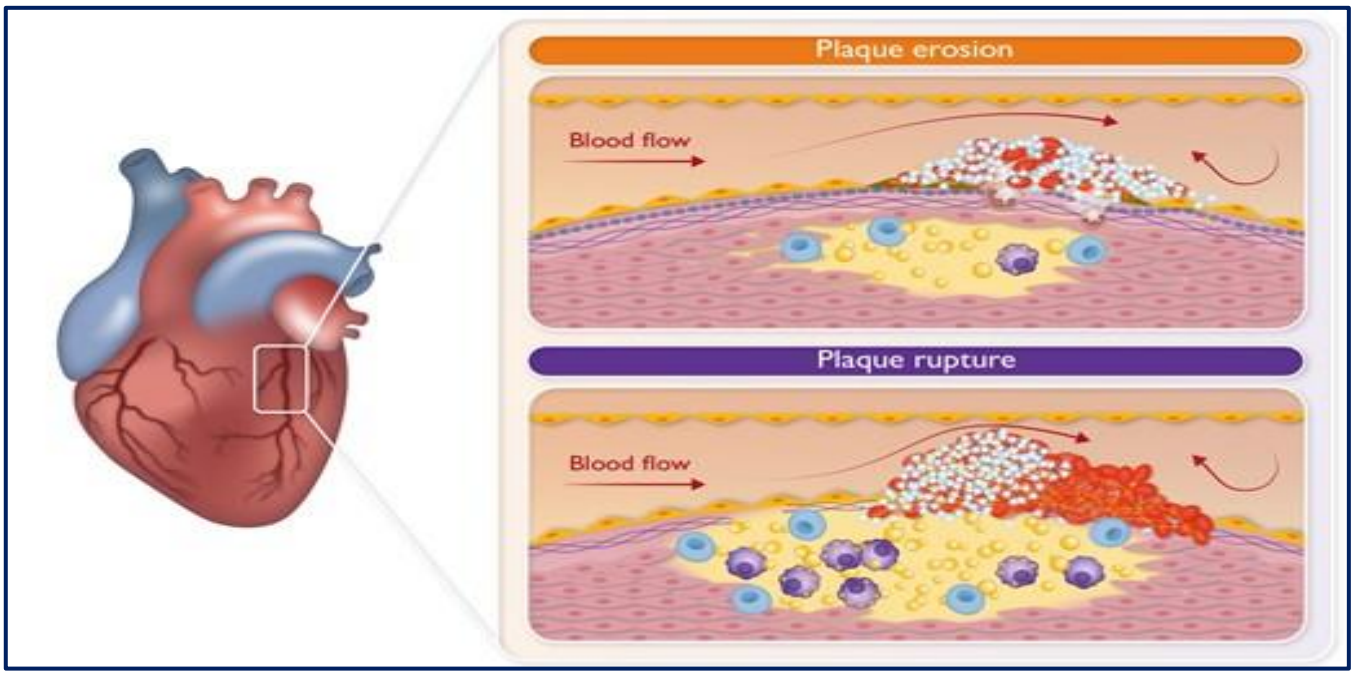


Figure (1-1): Illustration depicting the mechanisms of thrombus development caused by either erosion or rupture (Baaten et al., 2024).

C. Non-Atherosclerotic Causes

The third mechanism occurs due to non-atherosclerotic factors, without the presence of evident thrombosis. These factors include coronary vasospasm, spontaneous coronary artery dissection (SCAD), microvascular dysfunction (MB), stress-induced cardiomyopathy (Takotsubo syndrome). and coronary artery embolism caused by thrombus originating from another part of the body, leading to obstruction (Waterbury et al., 2020).

1.2.1.3. Risk factors

Risk factors for coronary artery disease are divided into modifiable and non-modifiable risk factors

A) Modifiable risk factors: The impact of modifiable risk factors for coronary heart disease (CHD) on prognostic performance is rather limited. However, it is important to note that the elimination or effective management of these individual factors could result in significant reductions in the occurrence of CHD events

within the whole population (Pencina et al., 2019). Maintaining a healthy weight, quitting smoking, and controlling blood pressure, cholesterol, and glucose levels can help prevent a large portion of the burden associated with CVD (Peters et al., 2019).

- **Hypertension:** The cardiovascular system experiences increased workload as it tries to propel blood across the vasculature. According to the World Health Organisation (WHO), around 62% of cardiovascular diseases (CVDs) and 49% of ischemic heart illnesses can be attributed to elevated blood pressure (BP) on a global scale Hypertension has always been recognised as a significant risk factor for the development of heart disease, mostly due to the oxidative and mechanical stress it imposes on the artery wall (Malakar et al., 2019). Hypertension was the second-leading modifiable CVD risk factor after cigarette smoking in the United States, accounting for more CVD Deaths than any other (Whelton et al., 2018).

- **Hyperlipidemia**: Hyperlipidemia is widely recognized as the second most prevalent risk factor associated with the development of ischemic heart disease (Brown et al., 2020). Lipid disorders, particularly those characterized by increased levels of cholesterol and low-density lipoprotein C (LDL-C) (Zhong et al., 2017). Reduced levels of HDL, a lipid that carries extra cholesterol from peripheral organs to the liver for storage or metabolism (Beverly and Budoff, 2020). are significant contributors to the development of cardiovascular diseases (CVDs) and ischemic stroke (Zhong et al., 2017). Dyslipidemia has been demonstrated as an independent predictor of numerous cardiovascular and cerebrovascular events on a global scale (Alloubani et al., 2021). To stop coronary heart disease from getting worse, it is essential to manage cardiovascular risk factors like hyperlipidemia over the long term (Gitt et al., 2023). Dyslipidemia and diabetes have a significant effect on the severity of

coronary artery disease (CAD). Therefore, the presence of these factors in patients with unstable angina classifies them as being in the high-risk category (Bhatia, 2023).

- **Diabetes mellitus:** Diabetes mellitus is widely recognised as a significant risk factor for the development of atherosclerotic coronary artery disease (CAD) (Hajar, 2017).

  The progression of arteriosclerosis is observed to occur at an earlier stage and to a greater degree in individuals with diabetes compared to those without diabetes (As in the figure (1-2)). Type 2 Diabetes mellitus (T2DM) and hyperlipidemia are well recognised as the primary risk factors. associated with Coronary Artery Disease (CAD).Research findings indicate that individuals diagnosed with type 2 diabetes mellitus (T2DM) exhibit an elevated risk of developing coronary artery disease (CAD), which is significantly greater when compared to individuals without T2DM (Yu et al., 2023). The incidence of CAD and heart failure is increased even in prediabetes (Cai et al., 2021, Cai et al., 2020). Compared to patients with T1DM, individuals with T2DM showed more non-calcified plaques and more severe Atherosclerotic Cardiovascular Disease (ASCVD) (Eckel et al., 2021).

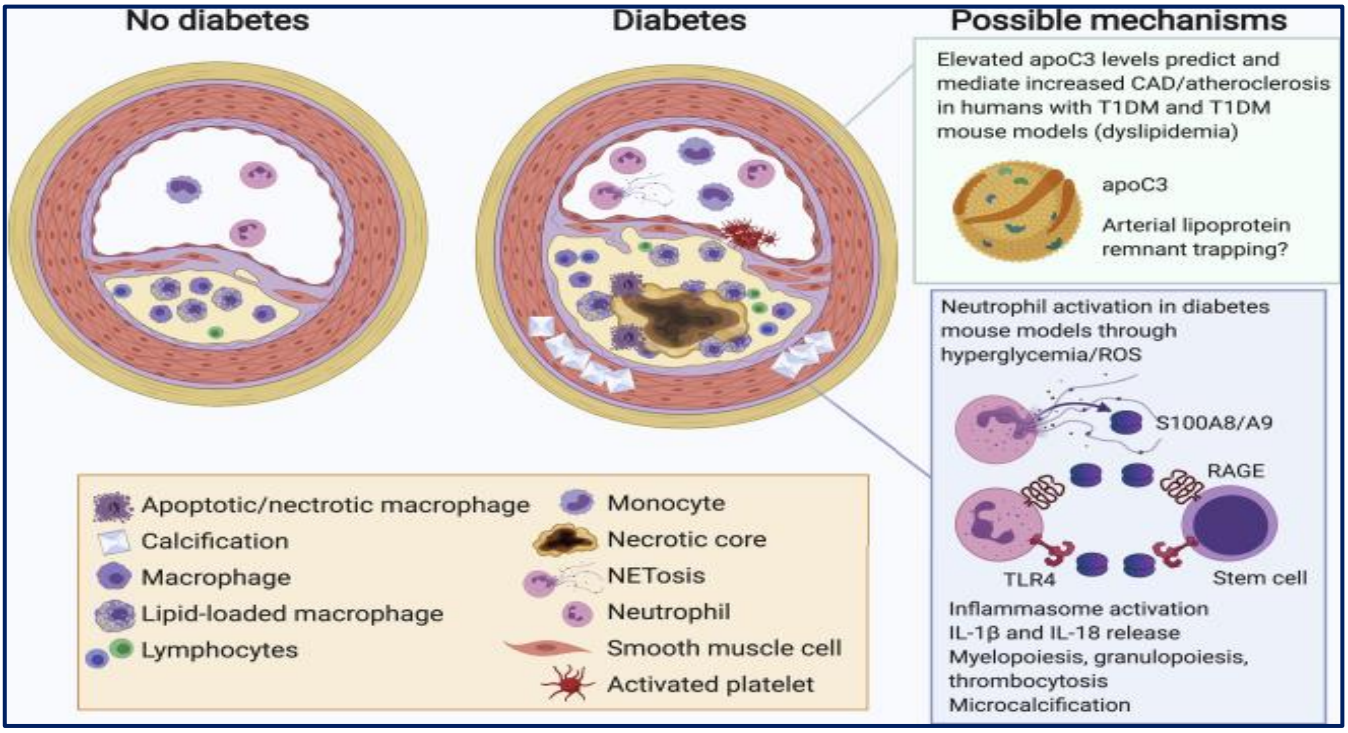


Figure (1-2): Morphological features of lesions of atherosclerosis in diabetes and non-diabetes (Eckel et al., 2021).

- **Physical activity:** may contribute to a reduction in the risk of coronary heart disease by around 20-30%. When examining different forms of physical activity, it is evident that those engaging in various household tasks may not receive sufficient cardiovascular protection. Regular physical activities such as walking, climbing stairs, swimming and cycling have been established as effective measures for reducing the risk of myocardial infarction (Mritunjay and Ramavataram, 2021). Regular physical activity has been shown to reduce the risk for CVD through a variety of mechanisms (Stanner et al., 2019).

- **Obesity:** Obesity has emerged as a significant worldwide health concern due to its widely known health risks and large increase in prevalence. (Gerdts and Regitz-Zagrosek, 2019).

Obesity, defined as a body mass index (BMI) exceeding 30 Kg/m^2 (or 28 Kg/m^2 in the Asian population). Obesity has been observed to influence the probability of developing ischemic heart disease, cardiac arrhythmias, and heart failure (HF) through many processes. The presence of an abundance of adipose tissue results in insulin resistance, inflammation, stimulation of the renin-angiotensin-aldosterone system (RAAS), and gradual alterations in the structure and electrical properties of the heart (Powell-Wiley et al., 2021). Obesity is a significant risk factor that contributes to the increased morbidity and mortality associated with cardiovascular diseases (CVDs)(Sutanto et al., 2021).

- **Smoking:** The act of tobacco smoking remains a significant risk factor for the development of cardiovascular disease (CVD) and stands as the primary preventable cause of mortality on a global scale (As in the figure(1-3)) (Kondo et al., 2019)**.** Smoking is responsible for 10% of all cardiovascular diseases (CVDs). The metals included in cigarette smoke are essential contributors to the harmful effects on the vascular endothelium. They have the ability to expedite processes that result in oxidative stress, which in turn causes damage and inflammation. These are the primary factors responsible for non-communicable chronic illnesses such as cardiovascular diseases, malignancies, degenerative diseases, and aging (Gallucci et al., 2020). Quitting smoking has the potential to be the most effective lifestyle intervention in preventing future cardiovascular events (Getz et al., 2023). The act of smoking has been demonstrated to result increase levels of coronary atherosclerosis, a condition that may be accountable for the elevated likelihood of developing hypertension, coronary heart disease, and atrial fibrillation. Consequently, this could perhaps explain why present smokers exhibit a greater occurrence of heart failure (Okorare et al., 2023).

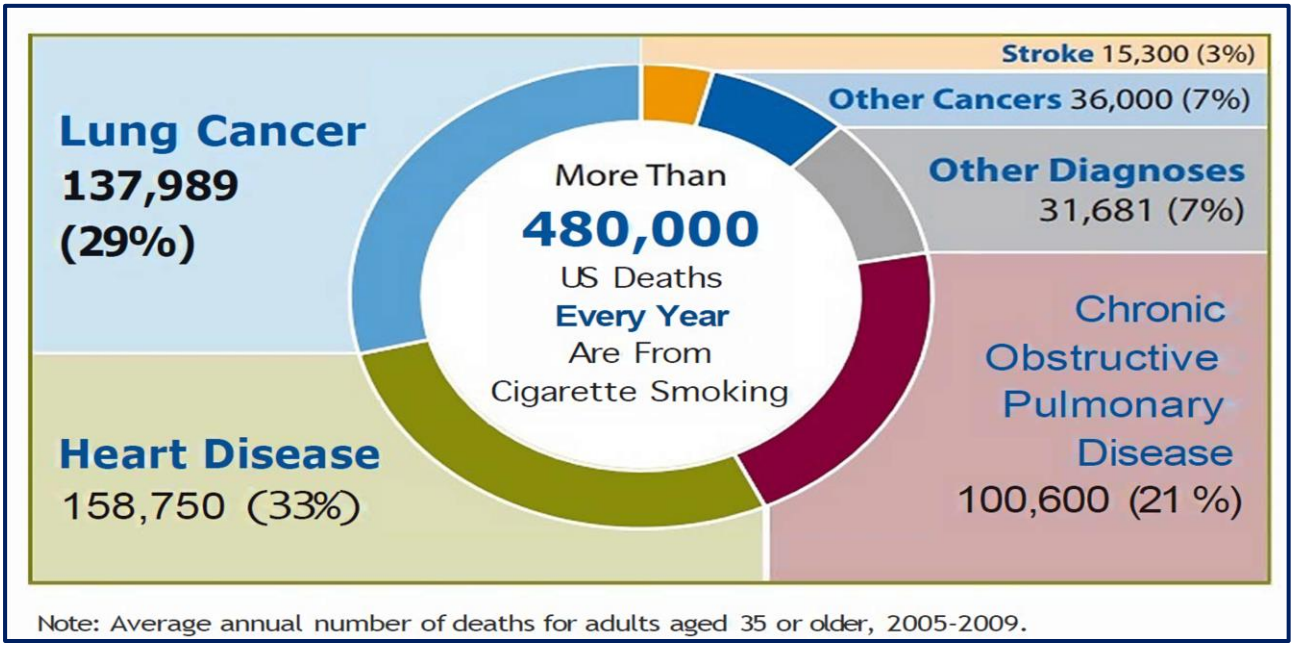


Figure (1-3): Annual number of deaths from cigarette smoking (Okorare et al., 2023)

- **Quality of diet:** The influence of a healthy diet on atherosclerotic cardiovascular disease (ASCVD) and its associated risk factors is significant (Arnett et al., 2019). The impact of lifestyle, particularly bad food habits, is a significant component in the onset of cardiovascular disease (CVD) and its related risk factors such as high cholesterol levels, hypertension, and type 2 diabetes mellitus, Conversely, intake healthy food and following a balanced diet might lead to positive results for cardiovascular health. (Bechthold et al., 2019). A healthy diet can protective human health from cardiovascular disease. A diet high in fruits, vegetables, low-fat dairy products, as well as reducing soda and sodium chloride intake and getting enough K^{+}, Ca^{2+}, and Mg^{2+}, vitamin C, and omega-3 fatty acids, can all help avoid CVD, These dietary lifestyles have a positive impact on the prevention of CVDs (Sharifi-Rad et al., 2020).

B) Non-modifiable Risk Factors: Knowing the non-modifiable risk factors associated with the development of coronary artery disease (CAD) is essential for effective disease management and the reduction of mortality rates**.**

- **Age:** Age is considered a non-modifiable risk factor. Previous research conducted among adult and elderly populations has indicated a positive correlation between age and the likelihood of having coronary artery disease (CAD), which can be attributed to elevated levels of serum cholesterol and high blood pressure (Raza et al., 2023)**.** CAD becomes more common in both males and females after the age of 35. The combined incidence of coronary artery disease (CAD) in individuals of both genders, aged 40 years and above, is estimated to be 49% for men and 32% for women (Brown et al., 2020).
- **Sex:** CVD has historically been seen as a health condition that impacts the male population. Although men often have higher rates of CVD at different ages, the overall likelihood of having CVD during their lifetime is similar for both women and men. (Peters et al., 2019). Moreover, available information indicates that CVD rates exhibit variations not just among genders but also within age and racial cohorts within the same gender (Benjamin et al., 2018, Peters et al., 2019). The collective burden of risk factors associated with cardiovascular disease was shown to be relatively lower in women than in males, regardless of the economic level of the countries they reside in, their geographical location, or whether they have a history of cardiovascular disease. This means that women may have a lower risk of developing cardiovascular disease compared to men (Walli-Attaei et al., 2020). Studies have demonstrated that estrogen plays a significant role in cardiovascular health and illness. is a potential cardio protectant that can be used to treat heart disease in women (Bajelan et al., 2019)

- **Family History:** Prior research has demonstrated that the presence of one or more first-degree relatives with coronary artery disease (CAD) is associated with a three-to six-fold elevation in the likelihood of getting CAD,The prevention of the disease can be achieved through early identification of families with a positive history of CAD (Raza et al., 2023). Family history also plays a crucial role in the context of younger women, as evidenced by the fact that women under the age of 65 with a maternal history of MI face a fourfold increase in the risk of ACS compared to men of the same age or older women (Haider et al., 2020).

1.2.1.4. Diagnoses

The World Health Organisation states that the diagnosis of acute myocardial infarction (AMI) relies on the presence of two out of three criteria, namely distinctive chest pain, diagnostic electrocardiographic abnormalities, and elevation of serum biochemical markers (Mirza et al., 2018).

1.2.1.4.1. Clinical presentation

The primary indicator that triggers the diagnostic and therapeutic process in individuals with suspected ACS is acute chest discomfort, which is characterized by sensations of pain, pressure, tightness, and burning (Collet et al., 2021). Other symptoms like as perspiration, vomiting, upper abdominal pain, difficulty breathing, and fainting may also be experienced. Unusual manifestations consist of discomfort in the upper abdomen, symptoms similar to indigestion, and difficulty in breathing or weariness, unusual symptoms are frequently seen in elderly individuals, particularly in women, as well as in patients with diabetes, chronic renal illness, or dementia (Collet et al., 2020).

1.2.1.4.2. Physical examination:

While physical examination findings may not typically provide specific indications for diagnosing acute coronary syndromes, a thorough evaluation of

the patient is crucial for promptly assessing immediate risks, detecting potential hemodynamic collapse, and identifying mechanical complications resulting from myocardial infarction (Bergmark et al., 2022). A physical examination can detect indications of chest discomfort that are not related to coronary issues, such as pulmonary embolism, acute aortic syndromes, pericarditis, or aortic stenosis. It can also indicate extra cardiac conditions including pneumothorax, pneumonia, or musculoskeletal illnesses. Within this context, the occurrence of chest discomfort that can be replicated by applying pressure to the chest wall exhibits a somewhat elevated negative predictive value for Acute Coronary Syndrome (ACS) (Byrne et al., 2023).

1.2.1.4.3. Diagnostic tools

A. Electrocardiogram (ECG)

The electrocardiogram (ECG) is a commonly used diagnostic tool for assessing acute myocardial ischemia and/or infarction. It has several advantages, including its widespread utilization, low costs, non-invasive nature, and easy accessibility (Zegre-Hemsey et al., 2019). The initial stage of assessment involves doing an electrocardiogram (ECG), which aids in distinguishing between STEMI and NSTE-ACS (Felix et al., 2024) The utilization of early and fast electrocardiogram (ECG) testing is recommended for all individuals who exhibit symptoms of chest pain, Females may encounter non-typical symptoms such as stomach discomfort or lightheadedness, whereas older individuals commonly experience dyspnea as a manifestation of myocardial infarction. Each of these presentations should elicit the need for electrocardiogram (ECG) testing (Mechanic et al., 2023). Individuals experiencing acute chest discomfort without persistent ST-segment elevation, commonly referred to as NSTE-ACS, The individual may display electrocardiogram (ECG) alterations, such as temporary elevation of the ST-segment, persistent or temporary depression of the ST-segment, inversion of the T-wave, flattened T-waves, or a pseudo-normalization

of T-waves. Alternatively, the ECG may appear within normal parameters (Collet et al., 2021).

B. Biomarkers: Biochemical markers, either alone or in conjunction with other diagnostic methods, are crucial instruments for the identification, management, and monitoring of diseases. They can offer a non-intrusive evaluation of the likely outcome, advancement of the illness, and the emergence of severe life-threatening consequences (Homsak and Gruson, 2020).

- **high-sensitivity cardiac troponin**

In the field of pathophysiology, it is important to note that NSTEMI and UA exhibit distinct characteristics. Specifically, NSTEMI is characterised by myocardial cell necrosis, whereas UA does not include any myocardial cell injury or necrosis. The differential diagnosis between the two conditions mostly relies on indicators of myocardial injury, particularly cardiac troponin levels, with a particular focus on high-sensitivity cardiac troponin (Wang et al., 2023c) Cardiac troponins T and I appear in the serum early after the onset of AMI, reaching a peak after 12-48 h and remaining elevated for 4-10 days, with a sensibility close to 100% in detecting AMI at 6-12 h after acute chest pain onset (Lazar et al., 2022)

C. Echocardiography (Echo.)

When there is doubt about the diagnosis of ACS, using a transthoracic echocardiogram (TTE) can be helpful in identifying indications of current ischemia or a previous myocardial infarction (MI) , TTE can also be valuable in proposing alternate causes linked to chest discomfort, such as acute aortic illness or the presence of right ventricular (RV) symptoms in pulmonary embolism (PE) (Byrne et al., 2023).

D. Coronary computed tomography angiography (CCTA) :

Is a reliable diagnostic imaging technique used to examine patients who are suspected of having coronary artery disease (CAD) (Gulati et al., 2022).In addition to its capability to detect luminal stenosis, CCTA enables the visualization of plaque, which is not possible with conventional invasive coronary angiography (ICA) without intravascular imaging. Nevertheless, the practical usefulness of these characteristics can be restricted in routine use due to inconsistencies in observations, especially among less skilled CCTA practitioners (Salem et al., 2023). If people have been eliminated as potential cases or if the results of an ECG or hs-cTn test are equivocal, a coronary computed tomography angiogram (CCTA) can be used as a non-invasive alternative to invasive coronary angiography to rule out acute coronary syndrome (ACS) (Guedeney and Collet, 2020).

E. Cardiac magnetic resonance (CMR)

Cardiac magnetic resonance (CMR) imaging accurately identifies the anatomy and function of the heart, and can also evaluate myocardial perfusion and the extent of myocardial damage, is the preferred imaging test when the quality of echocardiographic images is insufficient for an accurate diagnostic evaluation. Cardiac magnetic resonance (CMR) enables the direct observation of areas affected by infarction, offering insights into scarring and the capacity to distinguish it from other types of cardiac damage, such as myocarditis, Hence, it is especially valuable in determining a diagnosis of acute myocardial infarction (AMI) when there is uncertainty in the diagnostic process (Byrne et al., 2023).CMR enables precise visualisation and quantitative evaluation of several conditions including valve regurgitation/stenosis, ventricular volumes, ventricular systolic function,paravalvular extension, pericarditis, and myocarditis (Leiner et al., 2020).

F. Cardiac Catheterization: Cardiac catheterization is the gold standard and most accurate modality to evaluate ischemic coronary heart disease. It is however an invasive procedure with associated complications. Not everyone is a candidate for the procedure, In the ACS setting, all STEMI patients and selected NSTEMI patients get an emergent cardiac catheterization. This procedure is done in a cardiac catheterization lab, is expertise dependent, and is done under moderate sedation. There is contrast exposure in the procedure which could cause serious allergic reactions and kidney injury (Komilovich, 2023).

1.3. cardiac risk scores

Various risk classifications have been created to assist doctors in assessing the level of danger based on clinical factors, in addition to their own clinical judgement. While no score is perfect, risk assessments are nonetheless regarded as valuable instruments in clinical decision-making (Chan Pin Yin et al., 2020).
Risk stratification is a crucial aspect in the evaluation of patients with NSTEACS, as it has a direct impact on the first therapeutic approach, research has demonstrated that the utilisation of multivariate models is a more accurate approach in predicting risk compared to relying only on clinical impression (Cedro et al., 2021). The diagnosis of NSTEMI presents some obstacles, including the diverse range of symptoms exhibited by patients upon presentation and the limitations in accurately diagnosing the condition only through electrocardiography (ECG), Thus, in order to enhance the identification and treatment of NSTEMI, it is advisable to utilise scoring systems such as the thrombolysis in myocardial infarction risk index (TIMI) and HEART (Kesgün et al., 2022).
The assessment of potential risks was crucial in guiding the decisions taken regarding therapy.When determining the level of risk upon admission, guidelines recommend that high-risk patients receive more intensive invasive treatment (Yanqiao et al., 2022).

1.3.1. Thrombolysis in Myocardial Infarction risk score for UA/NSTEMI (TIMI):

The Thrombolysis in Myocardial Infarction (TIMI) score is a recognised approach to assess the early risk of individuals with unstable angina/non-ST elevated myocardial infarctions (UA/NSTEMI)(Sawalha et al., 2021). TIMI risk score is an effective measure for predicting the likelihood of coronary circulation involvement in patients of chest discomfort (Rao and Agasthi, 2020). The TIMI score helps guide the decision-making process for intervention techniques, whether they involve surgery or other approaches(Bahall, 2021). There are seven criteria that can be used to evaluate the risk of death and other negative cardiac events (Rao and Agasthi, 2023) as shown below :

- Age of 65 years or above
- Presence of a minimum of three risk factors for coronary artery disease, such as diabetes mellitus, hypertension, hyperlipidemia, smoking, or family history.
- Prior medical record indicating the presence of coronary stenosis with a degree of 50% or higher.
- Presence of at least 2 episodes of angina within the 24 hours prior to the presentation
- Aspirin utilisation in the prior seven days
- ST-segment deviations of 0.05 mV or higher on the first ECG upon admission
- Increased levels of cardiac markers indicating tissue death in the bloodstream

Each element contributes a singular value to the TIMI risk score, rendering it a straightforward instrument that does not necessitate varying weights for each factor, A higher score indicates an increased probability of experiencing negative cardiac events and/or an elevated risk of death(Sawalha et al., 2021). Patients with

scores ranging from 0 to 2 were classified as low-risk groups, scores ranging from 3 to 4 were classified as medium-risk groups, and scores ranging from 5 to 7 were classified as high-risk groups(Yanqiao et al., 2022). The correlation between TIMI score and severity of CAD is vital, as it allows for risk stratification of patients with acceptable TIMI risk scores and low-risk symptoms of ACS without the need for invasive procedures. This technique is also cost-effective. The TIMI risk score was identified as the most accurate predictor for determining the degree of coronary artery disease (Niazi et al., 2023).

1.3.2. HEART risk Score for Major Cardiac Events

The primary concern for emergency physicians when dealing with patients who appear with chest discomfort is determining whether to release the patient or not. Consequently, numerous scoring systems have been devised to facilitate this process of decision making (Gur et al., 2021). The HEART score is a predictive tool used to categorise patients with nonspecific chest pain into low-, intermediate-, and high-risk categories for short-term Major Adverse Cardiac Events (MACE)(Kim et al., 2021). The HEART score, which stands for

- History
- ECG
- Age
- Risk factors (Diabetes mellitus, Smoking, Hypertension, Dyslipidemia, FH of CAD, Obesity)
- Troponin

Heart score was first developed in 2007 and is widely utilized (Tolsma et al., 2023).

The history component was assigned 2 points for symptoms that were highly suspicious, 1 point for symptoms that were moderately suspicious, and 0 points for symptoms that were slightly suspicious. The ECG component was assigned a score of 2 for substantial ST-segment depressions, a score of 1 for non-specific

repolarization abnormalities, left bundle branch block, or ventricular paced rhythm, and a score of 0 for a normal ECG. The age component awarded 2 points for patients aged 65 years or over, 1 point for patients aged 45 to 64 years, and 0 points for patients below 45 years of age. The risk factors component awarded 2 points for the presence of three or more risk factors, 1 point for one or two risk factors, and 0 points for the absence of risk factors (Camaro et al., 2023). The troponin component score has consistently been determined based on the 99th percentile value of the assay. no points were granted if the levels of troponin T or I were equal to or below the 99th percentile. A score of 1 point would be assigned if the level was within a range that was one to three times higher than the 99th percentile. If the level surpassed or equaled three times the value at the 99th percentile, allocate 2 points (Tolsma et al., 2023).

Each variable is assigned a value ranging from 0 to 2 points, resulting in a cumulative score of 10 points. A number ranging from 0 to 3 suggests a low risk level, while a score between 4 and 6 indicates a medium risk level. A score between 7 and 10 indicates a high danger level(Huang et al., 2021). The low-risk patients were classified as suitable and secure for discharge from the emergency department without further cardiac assessment or hospital admission. Conversely, a higher score was linked to a greater rate of major adverse cardiac events (MACE) and necessitated extra evaluation and/or intervention (Brady and de Souza, 2018). The angiographic extent of coronary artery disease (CAD) showed a significant correlation with the TIMI, GRACE, and HEART risk scores. This highlights the importance of using these scores to assess the risk level and determine which patients would benefit the most from an early invasive treatment approach (Bhatia, 2023).

1.3.3. Reynolds risk Score (RRS) :

The Reynolds score is a predictive tool used to assess the likelihood of experiencing a heart attack, stroke, or other major cardiovascular event during the following ten year (Khandia et al., 2021)**.** The score incorporates the following variables (Gaasch et al., 2020):

- gender
- age
- smoking status
- systolic blood pressure
- total cholesterol
- HDL cholesterol
- (high sensitivity) CRP
- family history

Consequently, the search for biomarkers of inflammation that could greatly enhance the evaluation of cardiovascular disease (CVD) risk has led to the confirmation of an additional CVD risk score. namely the Reynolds Risk Score (RRS). Specifically, the stated score incorporates the parental history of myocardial infarction or stroke, together with high sensitivity C-reactive protein (hsCRP) which is now the most well-established inflammation biomarker (Klisić et al., 2018). Emerging scoring systems such as the Reynolds risk score emphasise the fundamental role of underlying inflammation in the development of coronary artery disease (CAD) in young individuals (Abhishek, 2019). The population was categorised into subgroups based on their level of risk, which included low, medium, and high risk groups,The three categories are defined as follows: RRS less than 5%, RRS between 5% and 10%, and RRS equal to or greater than 10% (Klisić et al., 2018).

1.4. Soluble Suppression of Tumorigenicity 2 (sST2):

Suppression of Tumorigenicity 2 (ST2) shows great potential as a biomarker for cardiovascular disease(van den Berg et al., 2022).

(ST2) is a constituent of the Interleukin-1receptor family (IL-1) and has two significant isoforms, specifically ST2 ligand (ST2L) and soluble ST2 (sST2). ST2L is a receptor that spans across the cell membrane, whereas sST2 is a receptor that is soluble and can be found in the bloodstream (Ip et al., 2021). The cognate ligand of ST2 is interleukin-33 (IL-33), a cardiac fibroblast protein released by stromal cells in cardiac and extra cardiac tissues. at the cardiac level, the ST2L/IL-33 interaction initiates a complex cardio protective biochemical cascade (Meijers et al., 2021). This inhibits the enlargement of heart muscle cells, programmed cell death, and the formation of scar tissue in the heart, ultimately leading to enhanced heart function. Yet, when the heart experiences harm or physical strain, cardio myocytes and cardiac fibroblasts release sST2. This substance competes with ST2L for the IL-33 binding site, counteracting the beneficial effect on the heart and leading to the development of myocardial fibrosis and ventricular remodeling (As in figure (1-4)) (Riccardi et al., 2023). Thus, the sST2 has been shown to diminish the putative cardioprotective impact of IL-33/ST2L by functioning as a decoy receptor(Gu and Li, 2019).

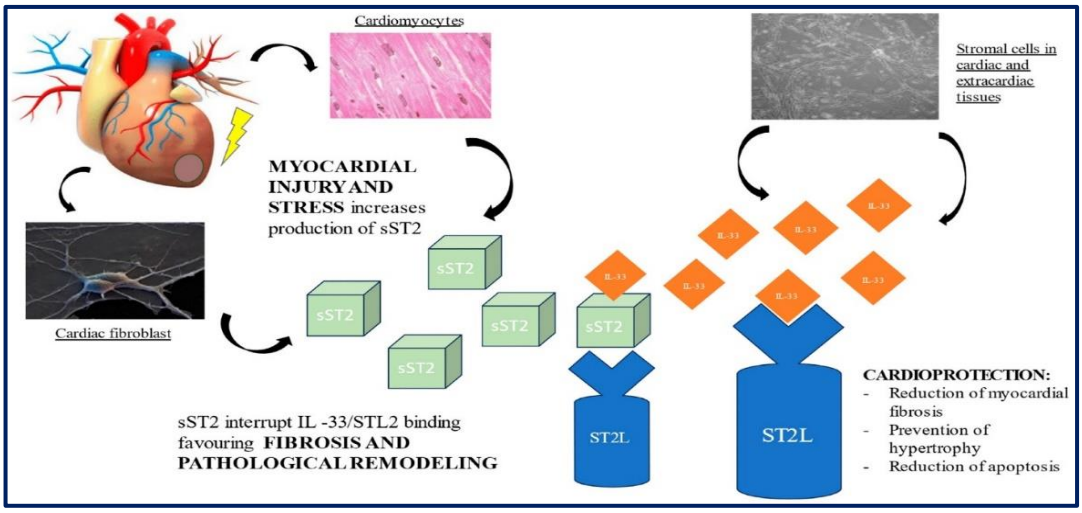


Figure (1-4): Pathological role of sST2 in promoting fibrosis and ventricular remodeling(Riccardi et al., 2023).

Given sST2's established role in inflammation, researchers conducted studies in different clinical settings to determine its potential as a predictive marker for patients with cardiovascular disease (Pál et al., 2023). The "inflammatory hypothesis" of atherosclerosis suggests that inflammation promotes the development, enlargement, and ultimately, the instability of atherosclerotic plaques, hence increasing the likelihood of cardiovascular events (Zhang et al., 2022). The IL-33-ST2L pathway has the potential to impede the progression of atherosclerosis by activating the immune response towards a T helper 2 and macrophage 2 phenotype. Conversely, elevated levels of sST2 could facilitate the formation of plaque by trapping IL-33 (Scicchitano et al., 2022). Consequently, in individuals with non-ST-elevation acute coronary syndrome, the concentration of serum sST2 could serve as a valuable indicator for predicting the vulnerability of plaques (Luo et al., 2021).

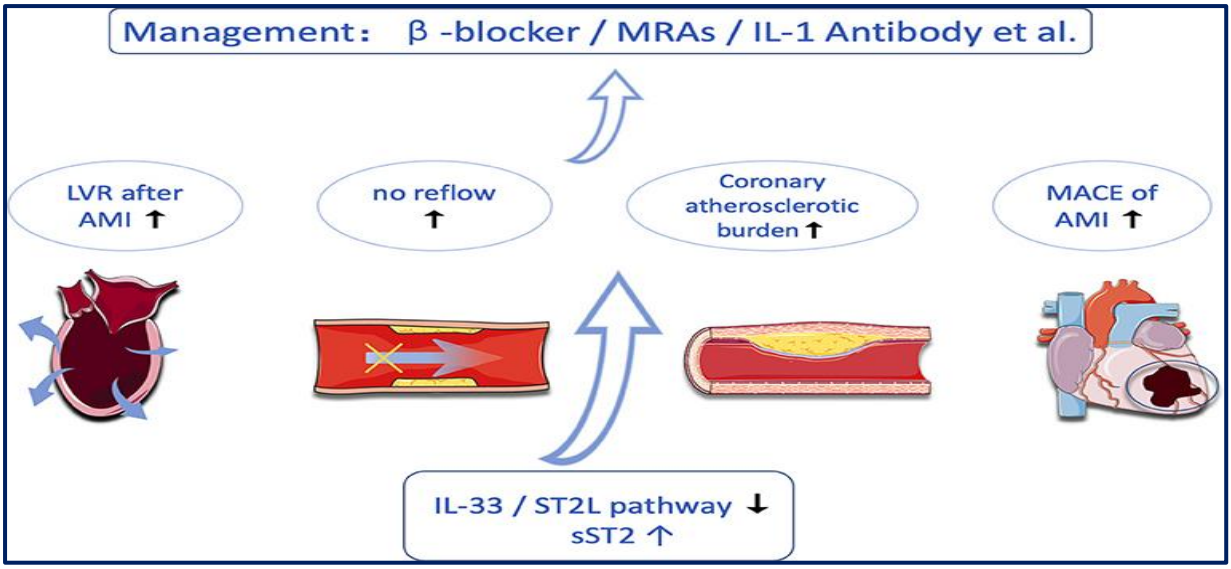


Figure (1-5): Role of sST2 in diseases of various systems (Zhang et al., 2022).

1.5. Insulin-like Growth Factor Binding Protein 2 (IGFBP-2):

The Insulin-like Growth Factor (IGF) system comprises of polypeptide hormones (IGF-1 and 2) and their surface receptors, namely Insulin-like growth factor receptors I and II (IGF-1R, IGF-2R), as well as six high-affinity IGF-binding proteins (IGFBPs; IGFBP-1 to -6) (Vaezi et al., 2023). IGFs share a similar structure to insulin and are found in various tissues throughout the body. IGF-1 is present not only in the bloodstream but also in arteries. Research has shown that IGF-1 has beneficial effects on the heart in cases of atherosclerosis (Wang et al., 2023b). Although insulin plays a fundamental role in the maintenance of normal blood glucose levels, however it is also acknowledged that IGF-I exerts complementary effects in glucose counter-regulation. Recently, important roles of other members of the IGF axis, particularly the IGF binding proteins, have become apparent in obesity and diabetes, and could potentially be exploited therapeutically (Haywood et al., 2019).

IGFBP2 is a member of the IGFBP family, can regulate the function and activity of IGFs by interacting with them in the bloodstream, and it also inhibits the connection between IGFs and IGF receptors. Additionally, it has the ability to attach to IGFs and induce either a growth-inhibiting or growth-stimulating impact that is depending on IGFs in certain types of cells(Wei et al., 2021).

The IGFBPs bind close to 99% of the circulating IGFs with high affinity, Binding to an IGFBP increases the half-life of IGF in the circulation (Allard and Duan, 2018). The binding of circulating IGFs with related proteins creates a reservoir of IGFs that is protected from degradation. However, these binding proteins can have a direct effect on cell function through mechanisms independent of IGFs (Yang et al., 2020).

The members of the IGFBP family exhibit a comparable structure and molecular organization, implying a similar mechanism of action. However, they are regulated differently and display diverse patterns of expression (Allard and Duan, 2018). IGFBP2 is mainly expressed in the heart and liver (Barutaut et al., 2020).

which is the second most prevalent IGFBP seen in the bloodstream (Rauzier et al., 2022). IGFBP2 functions in two primary ways: the IGF-dependent model, where it binds to and controls the availability of IGFs, and the IGF-independent model, where it works as a ligand by binding to receptors on the cell surface and extracellular matrix. Both models primarily work in the regulation of cell cycle, insulin, glucose metabolism (Boughanem et al., 2021).

IGFBP-2 expression is elevated during the developmental stages of the brain in the fetus and embryo, but experiences a notable decrease after birth (Harland et al., 2023). Elevated serum IGFBP-2 is associated with increased insulin sensitivity and a lower diabetes risk, but it also correlates with higher mortality rates in both diseased and healthy populations. High levels of IGFBP-2 are linked to greater cancer mortality risk and poorer cardiovascular outcomes. Its dual role may be due to its ability to exert both IGF-dependent and IGF-independent effects (Hjortebjerg et al., 2023).

IGFBP2 primarily exerts an inhibitory influence on the effects of IGF-1 in the circulation, this is due to its superior affinity for binding, in contrast to the affinity of IGF-1 for the IGF-1 receptor, IGF-1 inhibition plays a crucial role in the development of HF. Both human and experimental studies have shown IGF is cardio protective by reducing the effects of the renin angiotensin system, On the other hand, increased levels of IGF-1 are linked to enhanced cardiac remodeling and function following a heart attack. Therefore, increased levels of IGFBP2 may be linked to the suppression of IGF-1,leading to unopposed effects of renin angiotensin, and ultimately affecting left ventricular (LV) dysfunction (Barutaut et al., 2020).

1.6. Aim of the study

This study has some goals to achieve

1. Assessment of serum levels of soluble ST2 (sST2) and insulin-like growth factor binding protein 2 (IGFBP2) in non-ST segment elevation acute coronary syndrome (NSTE-ACS)

2. Association of Soluble suppression of tumorigenicity 2 (sST2) and Insulin-like Growth Factor Binding Protein 2 (IGFBP-2) with TIMI score , Reynolds score and heart score in patient with unstable angina/ Non-ST-Elevation Myocardial Infarction (NSTE-ACS)

3. Study the possibility of utilizing sST2 and IGFBP2 as substitutes for the TIMI score, Reynolds score, and heart score in categorizing individuals into low, medium, and high risk groups.

Chapter Two Materials and Methods

2. Materials and Methods.

2.1. Study Design and Ethical Approval.

A case-control study was carried out in the Karbala Center for Cardiac Diseases and Surgery and privet outpatient clinic. The participants were gathered over the duration spanning from October 2023 to January 2024. The study protocol was approval by the Ethical Committee of Karbala college of medicine and, Karbala Health Department. routine patient consent was obtained from patients or the or the patient's relatives.

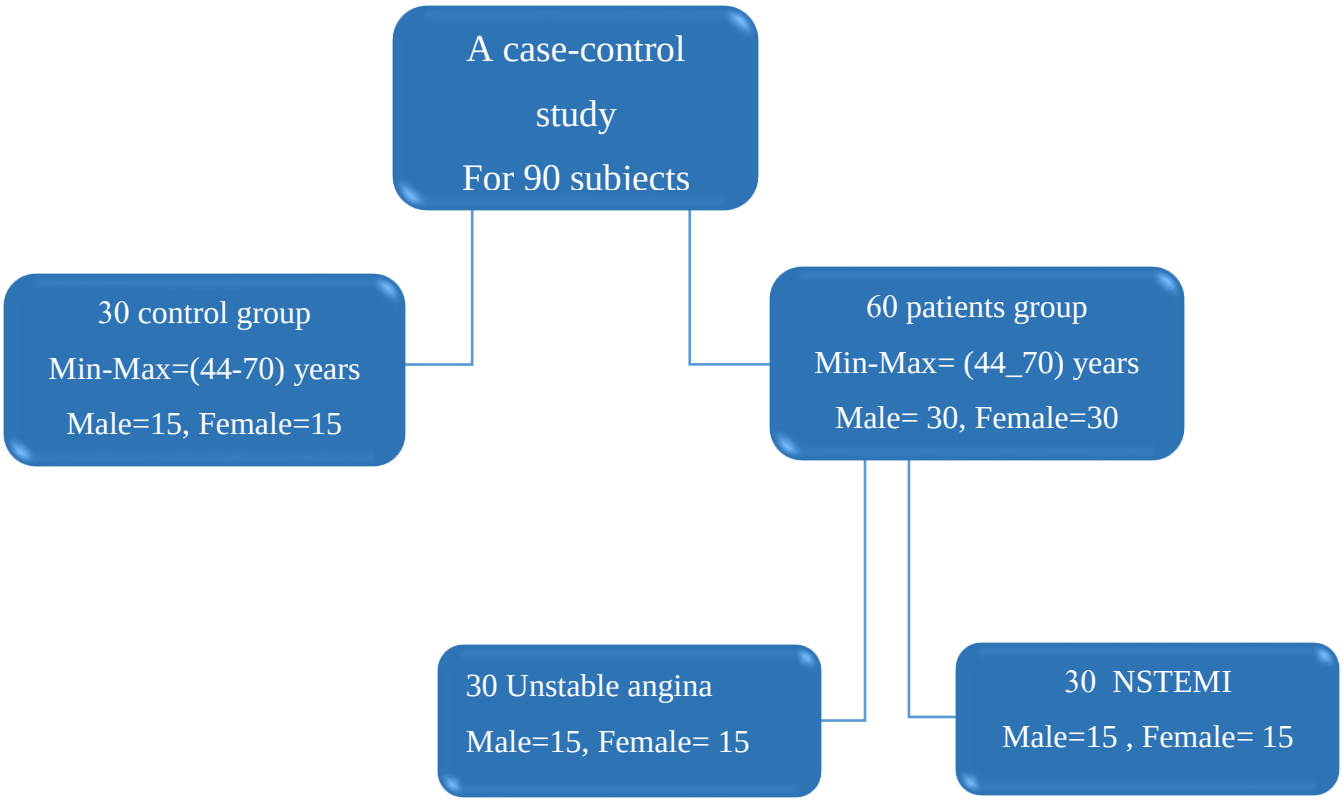


Figure (2-1): Study design flowchart

2.2. Subjects

2.2.1. Patients

The research involved 60 patients. aged between 44 -70 years old. These patients were divided into two subgroups: 30 with Non-ST segment elevation myocardial infarction (NSTEMI), 30 with Unstable angina (UA), as shown in fig (2-1). They were evaluated and diagnosed by specialized doctors. The patients were recruited from Karbala Heart Center and private outpatient clinic in the city of Karbala. All patients underwent 12 lead ECG A particular questionnaire form including descriptive information was designed and filled by each patient. The questionnaire included three sections. The first section was comprised of data collected from the patient, including age, gender, smoking status, current treatment, family history of premature coronary heart disease, physical activity, ≥ 3 risk factors for coronary artery disease (CAD), known CAD, aspirin use (in past 7 days), history of acute coronary syndrome (ACS), and severe angina. The second section consisted of a physical examination measuring SBP and DBP, height, and weight. The third section involved basic electrocardiographic parameters such as ST deviation and EKG changes, as well as blood biochemical tests like sST2, IGFBP2, Pro_BNP, Hs-Troponin I, lipid profile test, hs-CRP, and creatinine.

Inclusion Criteria

Patients with NSTEMI and UA diagnosed according to the history, clinical examination, electrocardiogram (ECG) and serum Troponin test .

Exclusion Criteria

Patients with acute and chronic kidney disease, heart failure, liver disease.

2.2.2. Control

A control group of 30 subjects (15 male and 15 female) aged between 44 -70 years old as shown in fig (2-1), with no history of ischemic heart diseases acute and chronic or heart failure and acute kidney injury.

2.2.3. Sample Collection

five milliliters of venous blood were collected from the individuals present at the mentioned places. The blood sample was transferred into a plain gel tube and allowed to coagulate at room temperature for approximately one hour. It was then separated using a centrifuge operating at a speed of 3000 xg for a duration of 20 minutes. The serum samples were divided into three Eppendorf tubes and kept at a temperature of -20 °C in the deep freezer until a time of analysis. In order to prevent repeated freezing and thawing cycles, a single tube was utilised for the measurement of serum pro-BNP, serum sST2, and serum IGFBP2. This measurement was conducted using the enzyme-linked immunosorbent assay (ELISA) technique. A single Eppendorf tube was utilized to quantify the lipid profile, creatinine, Hs-Troponin I and Hs-CRP levels utilizing an Auto analyzer chemical system.

2.3. Instruments

Table (2-1) The instruments used in the study.

No.	instruments	Country
1	Centrifuge	Germany
2	Deep Freeze	Korea
3	Autoanalyser Chemistry (AFLO)	China
4	ELISA system (Bio Tek)	USA
5	Mini VIDAS	French
6	Abbott Architect c4000	USA
7	Incubator (Binder)	Germany

2.4. Tools, materials and and Kits:

Table (2-2) Tools and materials used in the study.

No.	Tools and Materials	Country
1	pipette (100-1000µl)	DRAGON LAB/ USA
2	Micropipette (10-100µl)	DRAGON LAB/ USA
3	Gilson Tips,1000µl (blue)	China
4	Gilson Micro-tips, 100µl	China
5	Eppendorf Tubes 1.5ml	China
6	Eppendorf Tube Racks	China
7	Gel tubes	China
8	Syringe	China
9	Gloves	China
10	Tourniquet	China

Table (2-3): kits that are used in this research.

No.	Name of kit	Company	Country
1	sST2 Kit	BT LAB	China
2	IGFBP2 Kit	BT LAB	China
3	Pro_BNP	BT LAB	China
4	Total Cholesterol Kit	GIESSE	Italy
5	HDL-Cholesterol Kit	GIESSE	Italy
6	LDL-Cholesterol Kit	GIESSE	Italy
7	Triglycerides Kit	GIESSE	Italy
8	Creatinine	GIESSE	Italy
9	High sensitive_CRP	Abbott	USA
10	High sensitive Troponin I	bioMérieux	French

2.5. Methods

2.5.1. Calculation of Reynolds, TIMI, and Heart scores

The Reynolds, TIMI, and Heart scores were determined using a medical calculator system. This involved inputting the required clinical data into the score, and the system automatically computing scores for each patient.

2.5.2. Calculation of Body Mass Index

Weight in kilogram (kg) and height in meter (m) were recorded. Body mass index (BMI) was calculated by the following equation:

$$\textbf{BMI (kg/m}^2\textbf{) = weight / (height)}^2$$

Patients were divided into three categories: normal (BMI 18.5-24.9 kg/m^2), overweight (25-29.9 kg/m^2), and obese ($\geq$ 30.0 kg/m^2) (Donini et al., 2020).

2.5.3. Determination of Soluble Suppression of Tumorigenicty 2 (sST2) levels in Human serum by using Sandwich-ELISA Technique:

Test principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human SST2antibody. SST2 present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Human SST2Antibody is added and binds to SST2 in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated SST2 antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Human SST2. There action is terminated by addition of acidic stop solution and absorbance is measured at 450nm.

Reagents and materials

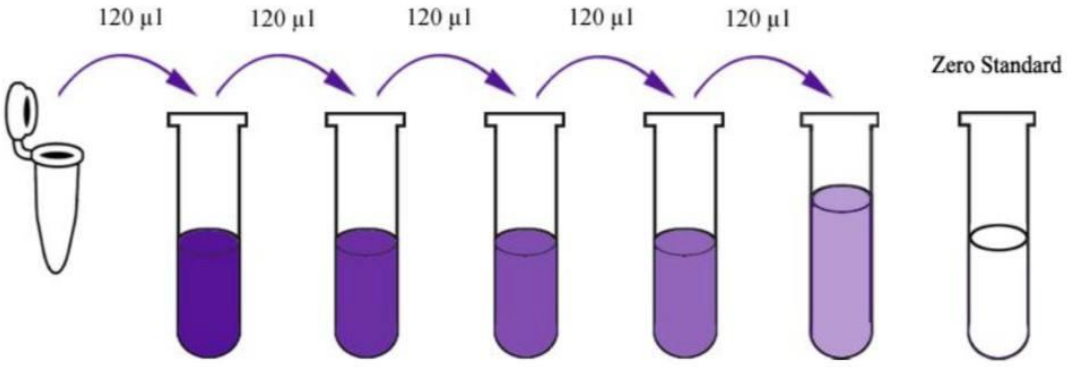
Table (2-4): Reagents provided with the ELISA kit .

Components	**Quantity (96T)**
Standard Solution (1280ng/L)	0.5ml x1
Pre-coated ELISA Plate	12 * 8 well strips x1
Standard Diluent	3ml x1
Streptavidin-HRP	6ml x1
Stop Solution	6ml x1
Substrate Solution A	6ml x1
Substrate Solution B	6ml x1
Wash Buffer Concentrate (25x)	20ml x1
Biotinylated Human SST2 Antibody	1ml x1
User Instruction	1
Plate Sealer	2 pics
Zipper bag	1 pic

Preparation of reagents:

Table (2-5): Dilution of Standards used for sSt2 assay.

640ng/L	Standard No.5	120µl Original Standard + 120µl Standard Diluent
320ng/L	Standard No.4	120µl Standard No.5 + 120µl Standard Diluent
160ng/L	Standard No.3	120µl Standard No.4 + 120µl Standard Diluent
80ng/L	Standard No.2	120µl Standard No.3 + 120µl Standard Diluent
40ng/L	Standard No.1	120µl Standard No.2 + 120µl Standard Diluent



Assay procedure

1. All reagents, standard solutions, and samples were prepared as instructed. All reagents were brought to room temperature before use. And The assay was performed at room temperature.
2. The number of strips required for the assay was determined. The strips were inserted in the frames for use. The unused strips were stored at 2-8°C.
3. 50µl standard was added to the standard well.
4. The 40µl sample was added to the sample wells and then the 10µl anti-SST2 antibody was added to the sample wells. Next, the 50µl streptavidin-HRP was added to the sample wells and standard wells. The plate was covered with a sealer and incubated for 60 minutes at 37°C.
5. The sealer was removed and the plate was washed 5 times with wash buffer. The wells were soaked with 300ul wash buffer for 30 seconds to 1 minute for each wash..
6. 50µl substrate solution A was added to each well, and then 50µl substrate solution B was added to each well. The plate was incubated covered with a new sealer for 10 minutes at 37°C in the dark.
7. 50µl Stop Solution was added to each well, and the blue color changed into yellow immediately.
8. The optical density (OD value) of each well was determined immediately using a microplate reader set to 450 nm within 10 minutes after the stop solution was added.

Calculation of results:

In Figure (2-2), A standard curve was constructed by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis. A best fit curve was drawn through the points on the graph. These calculations were performed with computer-based curve-fitting software and the best fit line was determined by regression analysis.

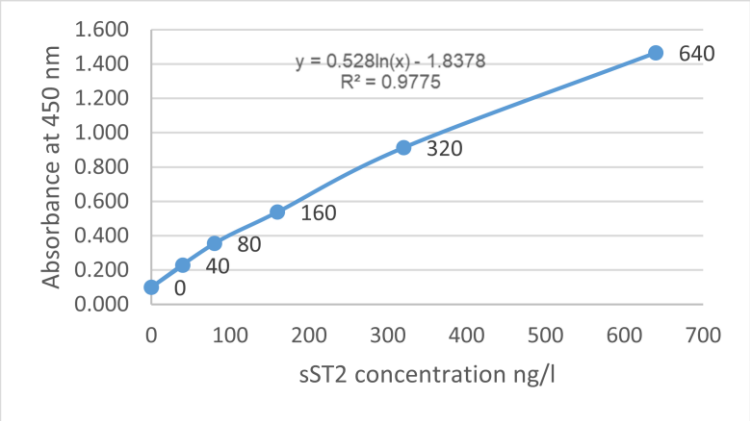


Figure (2-2): Standard curve of Human serum Soluble Suppression of Tumorigenicty 2 concentration (ng/L).

2.5.4. Determination of insulin-like growth factors binding protein 2 (IGFBP2) levels in Human serum by using Sandwich-ELISA Technique:

Test principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human IGFBP2 antibody. IGFBP2 present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Human IGFBP2Antibody is added and binds to IGFBP2 in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated IGFBP2 antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solutions then added and color develops in proportion to the amount of Human IGFBP2.The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Reagents and materials

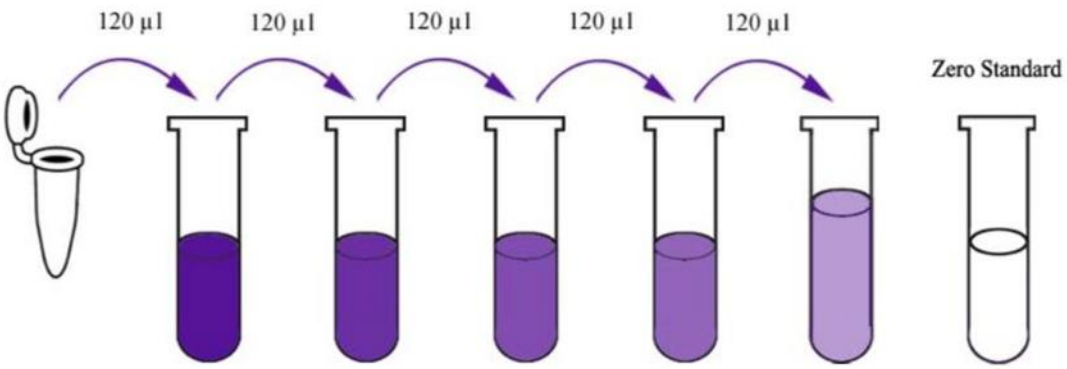
Table (2-6): Reagents provided with the ELISA kit.

Components	Quantity (96T)
Standard Solution (128ng/ml)	0.5ml x1
Pre-coated ELISA Plate	12 * 8 well strips x1
Standard Diluent	3ml x1
Streptavidin -HRP	6ml x1
Stop Solution	6ml x1
Substrate Solution A	6ml x1
Substrate Solution B	6ml x1
Wash Buffer Concentrate (25x)	20ml x1
Biotinylated Human IGFBP2 Antibody	1ml x1
User Instruction	1
Plate Sealer	2 pics
Zipper bag	1 pic

Preparation of reagents:

Table (2-7): Dilution of Standards used for IGFBP2 assay.

64 ng/ml	Standard No.5	120µl Original Standard + 120µl Standard Diluent
32 ng/ml	Standard No.4	120µl Standard No.5 + 120µl Standard Diluent
16 ng/ml	Standard No.3	120µl Standard No.4 + 120µl Standard Diluent
8 ng/ml	Standard No.2	120µl Standard No.3 + 120µl Standard Diluent
4ng/ml	Standard No.1	120µl Standard No.2 + 120µl Standard Diluent



Assay procedure

1. All reagents, standard solutions, and samples were prepared as instructed. All reagents were brought to room temperature before use. And The assay was performed at room temperature.
2. The number of strips required for the assay was determined. The strips were inserted in the frames for use. The unused strips were stored at 2-8°C.
3. 50µl standard was added to the standard well.
4. The 40µl sample was added to the sample wells and then the 10µl anti-IGFBP2 antibody was added to the sample wells. Next, the 50µl streptavidin-HRP was added to the sample wells and standard wells. The plate was covered with a sealer and incubated for 60 minutes at 37°C.
5. The sealer was removed and the plate was washed 5 times with wash buffer. The wells were soaked with 300ul wash buffer for 30 seconds to 1 minute for each wash.
6. 50µl substrate solution A was added to each well, and then 50µl substrate solution B was added to each well. The plate was incubated covered with a new sealer for 10 minutes at 37°C in the dark.
7. 50µl Stop Solution was added to each well, and the blue color changed into yellow immediately.

8. The optical density (OD value) of each well was determined immediately using a microplate reader set to 450 nm within 10 minutes after the stop solution was added.

Calculation of results

In Figure (2-3), A standard curve was constructed by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis. A best fit curve was drawn through the points on the graph. These calculations were performed with computer-based curve-fitting software and the best fit line was determined by regression analysis.

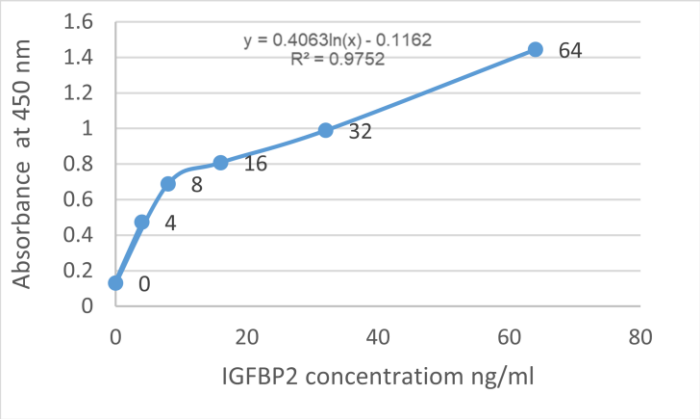


Figure (2-3): Standard curve of Human serum IGFBP-2concentration (ng/ml).

2.5.5. Determination of pro-brain natriuretic peptide (pro-BNP) levels in Human serum by using Sandwich-ELISA Technique:

Test principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human PRO-BNP antibody. PRO-BNP present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Human PRO-BNP Antibody is added and binds to PRO-BNP in the sample.

Then Streptavidin-HRP is added and binds to the Biotinylated PRO-BNP antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Human PRO-BNP. The reaction is terminated by addition of acidic to stop solution and absorbance is measured at 450 nm .

Reagents and materials

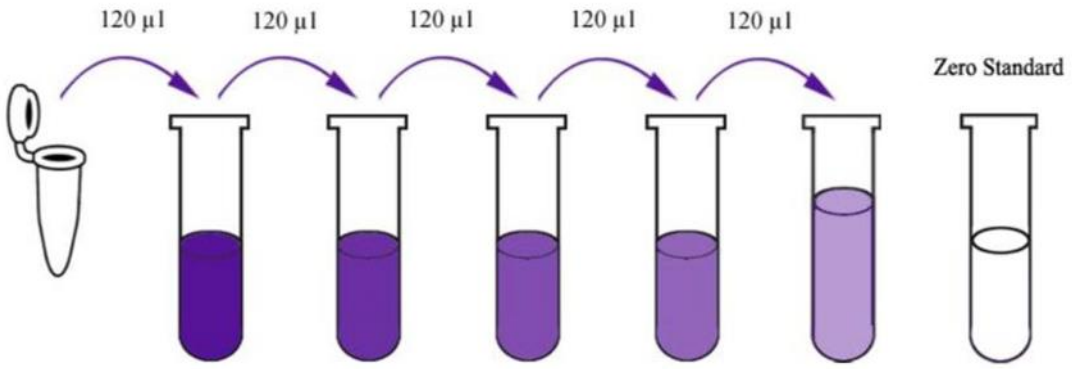
Table (2-8): Reagents provided with the ELISA kit.

Components	**Quantity (96T)**
Standard Solution (400 ng/ml)	0.5ml x1
Pre-coated ELISA Plate	12 * 8 well strips x1
Standard Diluent	3ml x1
Streptavidin -HRP	6ml x1
Stop Solution	6ml x1
Substrate Solution A	6ml x1
Substrate Solution B	6ml x1
Wash Buffer Concentrate (25x)	20ml x1
Biotinylated Human PRO-BNP Antibody	1ml x1
User Instruction	1
Plate Sealer	2 pics
Zipper bag	1 pic

Preparation of reagents:

Table (2-9): Dilution of Standards used for IGFBP2 assay.

200 ng/L	Standard No.5	120µl Original Standard + 120µl Standard Diluent
100 ng/L	Standard No.4	120µl Standard No.5 + 120µl Standard Diluent
50 ng/L	Standard No.3	120µl Standard No.4 + 120µl Standard Diluent
25 ng/L	Standard No.2	120µl Standard No.3 + 120µl Standard Diluent
12.5ng/L	Standard No.1	120µl Standard No.2 + 120µl Standard Diluent



Assay procedure

1. All reagents, standard solutions, and samples were prepared as instructed. All reagents were brought to room temperature before use. And The assay was performed at room temperature.
2. The number of strips required for the assay was determined. The strips were inserted in the frames for use. The unused strips were stored at 2-8°C.
3. 50µl standard was added to the standard well.
4. The 40µl sample was added to the sample wells and then the 10µl anti -PRO-BNP antibody was added to the sample wells. Next, the 50µl streptavidin-

HRP was added to the sample wells and standard wells. The plate was covered with a sealer and incubated for 60 minutes at 37°C.

5. The sealer was removed and the plate was washed 5 times with wash buffer. The wells were soaked with 300ul wash buffer for 30 seconds to 1 minute for each wash.
6. 50µl substrate solution A was added to each well, and then 50µl substrate solution B was added to each well. The plate was incubated covered with a new sealer for 10 minutes at 37°C in the dark.
7. 50µl Stop Solution was added to each well, and the blue color changed into yellow immediately.
8. The optical density (OD value) of each well was determined immediately using a microplate reader set to 450 nm within 10 minutes after the stop solution was added.

Calculation of results

In Figure (2-4), A standard curve was constructed by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis. A best fit curve was drawn through the points on the graph. These calculations were performed with computer-based curve-fitting software and the best fit line was determined by regression analysis.

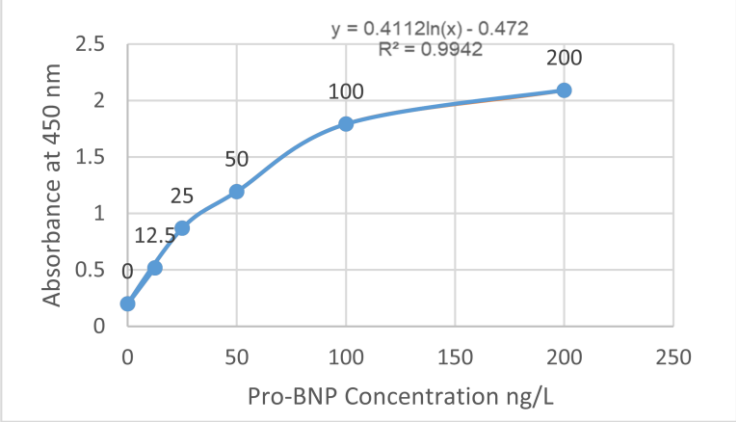


Figure (2-4): Standard curve of pro-BNP concentration (ng/L).

2.5.6. Determination of Lipid Profile

2.5.6.1. Determination of Total cholesterol level

Test principle

Esterified cholesterol is hydrolyzed into free cholesterol and fatty acid by cholesterol esterase (CHE). Cholesterol oxidase (CHOD) oxidizes the free cholesterol into cholesta-4-ene-3-one with the formation of hydrogen peroxide. In the presence of peroxidase (POD), hydrogen peroxide reacts with a derivative of phenol and 4-amino antipyrine to produce a colored complex whose color intensity is directly proportional to the total cholesterol concentration in the sample.

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

Reagent (A) Vol = 50/100/250/1000 mL	Buffer4- AAPCHE CHODPOD Derivative of phenol	100 mmol/L 1 mmol/L 300 U/L 300 U/L 1500 U/L 1 mmol/L
Standard Vol = 5 mL	Cholesterol	200 mg/L

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

Reagent Preparation: liquid reagent, bring to room temperature (15-25 C before use. Mixed 6.250 ml from reagent A and 0.125 from reagent B.

Procedure:

Concentrations of total cholesterol were measured by using autoanalyzer chemistry and the procedure for blank, standard, and sample measurement is demonstrated in Table (2-5). The mixture was prepared and incubated at 37°C for 5 minutes. The Sample reagent ratio was 1:100, and the absorbance of the sample (Ax) and the standard (As) was read against a blank reagent at 510 nm.

Table (2-11): Procedure of total cholesterol .

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

Calculation:

Serum/plasma:

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

2.5.6.2. Determination of Triglyceride concentration.

Principle

Triglycerides are(TG) hydrolyzed by lipoprotein lipase (LPL) to produce glycerol and free fatty acids. The glycerol participates in a series of coupled enzymatic reactions, in which glycerol kinase (GK) and glycerol phosphate oxidase (GPO) are involved and H_2O_2 is generated. The hydrogen peroxide reacts with TOOS and 4-Aminoantipyrine (4-AAP) to form a colored complex, whose color intensity is directly proportional to the concentration of triglycerides in the sample.

Table (2-12): Reagents used for triglycerides assay.

Reagent (A) Volume = 50/100/250/1000 ml	Good buffer Magnesium chloride ATP 4-AAP TOOS LPL (lipoproteinlipase) POD (peroxidase) GK (glycerol kinase)GPO	100 mmol/L 15 mmol/L 4 mmol/L 1 mmol/L 0.1 mmol/L 2500 U/L 1800 U/L 1000 U/L 5500 U/L
Standard Volume = 10 ml	Glycerol	200 mg/dl (2.28 mmol/l)

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

Reagents Preparation

Liquid reagent, bring to room temperature (15-25 C) before use. The light color of the reagent (<0.050 O.D.) due to air or light does not affect their operation.

Procedure

Concentrations of triglycerides were measured by using autoanalyzer chemistry and the procedure for blank, standard, and sample measurement is demonstrated in Table (2-8). The mixture was prepared and incubated at 37°C for 5 minutes. The Sample to reagent ratio was 1:100, and the absorbance of the sample (Ax) and the standard (As) were read against a blank reagent at 510 nm.

Table (2-13): Procedure of triglyceride.

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

Calculation*:*

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

2.5.6.3. Determination of High-density Lipoprotein Cholesterol concentration.

Test Principle

Specific polyanions in the first phase block the interfering lipoproteins (LDL, VLDL, chylomicrons), and a specific surface-active agent inhibits the coloration of VLDL, LDL, and chylomicrons in the second phase. The intensity of color produced is directly proportional to the HDL cholesterol in the sample

Table (2-14): Reagents used for high-density lipoprotein cholesterol assay.

Reagent (A) Volume = 90 mL	Good Buffer Polianions 4-AAP	100 mmol/L 1mmol/L 4 mmol/L
Reagent (B) Volume = 30	Cholesterol esterase Cholesterol oxidase Peroxidase HDAOS Detergent	800 U/L 500 U/L 1500 U/L 1 mmol/l 4 mmol/l

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

Reagent Preparation

Liquid reagents, bring to room temperature (15-25C) before use. Reconstitute the calibrator (included in the kit. 0026T) with 3.0 ml of distilled water.

Procedure

Table (2-15): Procedure of high-density lipoprotein.

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

Calculation:

HDL (mg/dl) = (Ax-Abx) / (Ac-Abc) × Calibrator Value

2.5.6.4. Calculation of Low-density Lipoprotein Cholesterol concentration.

Test Principle

When a sample is mixed with Reagent (A), the protecting reagent binds to LDL and protects LDL from enzyme reactions. Cholesterol esterase and cholesterol oxidase react with non-LDL lipoproteins (chylomicrons, VLDL and HDL). Hydrogen peroxide produced is decomposed by catalase. When Reagent (B) is added, the protecting reagent is removed from LDL and catalase is inactivated. In this second process, the enzymatic reactionis conducted solely on the LDL fraction and the hydrogen peroxide produced yields a color complex upon oxidase condensation with HDAOS [N-(2-hydroxy-3-sulfopropy!) 3,5-dimethoxyaniline] and 4-AAP in the

presence of peroxidase. Colour intensity is directly proportional to the amount of LDL cholesterol in the sample.

Table (2-16): Reagents used for Low-density Lipoprotein Cholesterol assay.

Reagent (A) LDL Volume 10 ml	Good Buffer HDAOS	20 mM 1mM
Reagent (B) LDL Volume 10 ml	Good Buffer Cholesterol esterase Cholesterol oxidase Peroxidase 4-AAP	20 mM 5.0 U/ml 1.0 U/ml 15 U/ml 3.0 U/ml

Reagent Preparation

Liquid Reagents, bring to room temperature (15-25°C) before use.

Procedure

Table (2-17): Procedure of Low-density lipoprotein.

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

Calculation:

LDL (mg/dl) = (Ax-Abx) / (Ac-Abc) × Calibrator Value

2.5.7. Determination of Creatinine concentration.

Test Principle

Creatinine reacts in an alkaline environment with picric acid forming a salt of a yellow-orange color. The intensity of the color that develops in a predetermined time interval is proportional to the amount of creatinine in the sample.

Table (2-18): Reagents used for creatinine assay.

Reagent (A) CREA Volume =50/100/250 ml	Picric Acid	29 mmol/l
Reagent (B) CREA Volume =50/100/250 ml	Buffer Sodium hydroxide	100 mmol/l 500 mmol/l

Reagent Preparation

Mix the Reagents (A) and (B) into equal parts. Stabilize the working solution 15 minutes before use. Use the necessary quantities depending on the number of analyzes to be carried.

The working solution is stable for 7 days at room temperature.

Procedure

Table(2-19): Procedure of creatinine.

Pipette	Sample(µl)	Standard(µl)
Reagent (A+B)	1000	1000
Sample	100	
Standard		100

Calculation:

Creatinine (mg/dl) = (Ax2-Ax1) / (As2-As1) × Standard Value

2.5.8. Determination of (High sensitivity-C reactive protein) levels in Human serum .

Test principle

MULTIGENT CRP Vario is a latex immunoassay developed to accurately and reproducibly measure blood CRP levels in serum and plasma. When an antigen-antibody reaction occurs between CRP in a sample and anti-CRP antibody, which has been adsorbed to latex particles, agglutination results. This agglutination is detected as an absorbance change (572 nm), with the rate of change being proportional to the quantity of CRP in the sample. Three different methods (High Sensitivity [CRP16], Standard [CRP32], and Wide Range [CRP48]) are available to cover a wide analytical measurement range.

Table (2-20): Reagents used for hs-CRP assay.

2 Reagent Kit Configurations

6K26-30	R1	2 *37 ml
	R2	2 *37 ml
6K26-41	R1	3 *86 ml
	R2	3 *86 ml

Reagent Preparation

Reagent is liquid, ready-to-use, 2 reagent kit.

Procedure

Step 1: 40(µl) from Sample was added to 100(µl) from Reagent 1 (Glycine Buffer).
Step 2: 100(µl) from Reagent 2 containing Antibody (anti-human CRP rabbit serum) was added to the sample/Reagent 1 mix.

Step 3: When the sample containing CRP was added to RI and mixed with the antibody in Reagent 2, insoluble aggregates were formed. The degree of agglutination was directly proportional to the concentration of CRP in the sample. This was determined by measuring the decrease of transmitted light caused by the aggregates at a wavelength of 572 nm.

Calculation:

Hs-CRP (mg/dl) = $(A2\text{-}A1)_{sample} / (A2\text{-}A1)_{calibration} \times$ CAL conc.= mg/L CRP

2.5.9. Determination of (High sensitive Troponin I) levels in Human serum.

Test principle

The assay principle combines a one-step enzyme immunoassay sandwich method with a final fluorescent detection (ELFA). The Solid Phase Receptacle (SPR®) serves as the solid phase as well as the pipetting device. Reagents for the assay are ready-to-use and pre-dispensed in the sealed reagent strips. All of the assay steps are performed automatically by the instrument. The sample is transferred into the wells containing alkaline phosphatase-labeled anti-cardiac troponin antibodies (conjugate). The sample/conjugate mixture is cycled in and out of the SPR® several times. This operation enables the troponin I to bind with the immunoglobulins, fixed to the interior wall of the SPR®, and the conjugate, to form a sandwich. Unbound components are eliminated during washing steps. Two detection steps are then performed successively. During each step, the substrate (4-Methyl-umbelliferyl phosphate) is cycled in and out of the SPR®. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methyl umbelliferone), the fluorescence of which is measured at 450 nm. The intensity of the fluorescence is proportional to the concentration of antigen present in the sample. At the end of the assay, the results are automatically calculated by the instrument in relation to two

calibration curves stored in memory corresponding to the two detection steps. A fluorescence threshold value determines the calibration curve to be used for each sample. The results are then printed out.

Reagents and materials

Table (2-21): Content of (hs- cTnI) kit.

60 TNHS Strips	STR	Ready-to-use.
60 TNHS SPR®s 2 x 30	SPR®	Ready-to-use.
TNHS Controls C1 Control 1 x 2 mL C2 Control 1 x 2 mL	C1 C2	Reconstitute with 2 mL of distilled water.
TNHS Calibrators S1 Calibrator 2 x 2 mL S2 Calibrator 2 x 2 mL	S1 S2	Reconstitute with 2 mL of distilled water.
TNHS Diluent 1 x 2 mL (liquid)	R1	Ready-to-use.

Procedure

1. The required reagents were removed from the refrigerator. They could be used immediately.

2. One "TNHS" strip and one "TNHS" SPR® were used for each sample, control, or calibrator that was tested. It was ensured that the storage pouch had been carefully resealed after the required SPR®s had been removed.
3. The test was identified by the "TNHS" code on the instrument. The calibrators had to be identified by "S1" and "S2", and tested in duplicate. The controls were identified by "C1" and "C2" and tested singly.
4. The calibrators and/or controls and/or samples were mixed using a vortex-type mixer (for serum or plasma separated from the pellet).
5. Before pipetting, it was ensured that the samples, calibrators, controls, and diluent were free of bubbles.
6. For this test, the test portion of the calibrators, controls, and samples was 200 µL.
7. The "TNHS" SPR®s and "TNHS" strips were inserted into the instrument. It was checked to ensure that the color labels with the assay code on the SPR®s and the Reagent Strips matched.
8. The assay was initiated immediately. All the assay steps were performed automatically by the instrument.
9. The vials were reclosed and returned to the required temperature after pipetting.
10. The assay was completed within approximately 20 minutes. After the assay was completed, the SPR®s and strips were removed from the instrument.
11. The used SPR®s and strips were disposed of into an appropriate recipient.

Calculation:

The results were automatically calculated by the instrument using two calibration curves which were stored by the instrument, and were expressed in ng/L (or pg/mL). The VIDAS® High sensitive Troponin I assay was standardized against a competitor's method. The VIDAS® High sensitive Troponin I assay results were displayed by the instrument from 1.5 to 40,000 ng/L.

Statistical Analysis

The Statistical Package for Social Sciences (SPSS) for Windows 10 (IBM SPSS 26.0, Chicago, IL, USA) program was used for the statistical assessments. Descriptive statistical approaches were used to analyze the data from each group. Scale variables were represented by their Median (Min-Max) and interquartile range (IQR), whereas values for categorical variables were reported as the number of occurrences (n) and \the corresponding percentage (%). The Shapiro-Wilk test was utilized to confirm the normality of the data distribution.

Additionally, an inferential statistical approaches are used to analyze the abnormal distribution, The Mann-Whitney U test was used to compare continuous variables between two groups. The Kruskal-Wallis test was employed to compare continuous variables among multiple groups. The correlation within the case study was evaluated by comparing biomarkers using the Spearman rank test.

The association between the analyzed factor were estimated using odds ratios (ORs) and 95% Confidence Interval Range which calculated by a non-conditional logistic regression.

Analytical statistical studies indicated significant differences in categorical variables among the parameters. All hypothesis tests with p-values less than 0.05 (two-sided) were deemed to have statistical significance.

The optimal threshold with high specificity and sensitivity for critical cases was detected using receiver operating characteristic (ROC) analysis

Chapter Three
Results

3. Results

3.1. Demographic Characteristics

The demographic and clinical characteristics of patients with NSTE-ACS, namely (NSTEMI, UA) and control groups were summarized in Table (3-1) and Figures (3-1), (3-2). The proportion of patients aged (40-50 year) was 11.7%, while the proportion of patients aged (51-61 year) was 48.3%. Patients aged 62 years and above account for 40% of the total. In the control group, the proportion of people aged 40–50 year was 46.7%, the percentage of people aged 51–61 year was 30%, and the percentage of people aged 62 years and above is 23.3%. The sex distribution among. the studied groups was evenly split, with 50% male and 50% female. The patients' BMI categories are distributed as follows: 28.3%were classed as having a normal BMI, 33.3% were classified as overweight, and 38.3%were classified as obese. The control group consists of individuals with the following proportions: 50% have a normal BMI, 26.7% were overweight, and 23.3% were obese. The patients' medical history was obtained by a questionnaire, revealing that around 20% of them were smokers, 60% had diabetes mellitus (DM), 73.3% had hypertension, 21.7% had a family history of premature coronary artery disease (CAD), 61% had known CAD and 60% engaged in regular physical activity.

Table (3-1): Demographic and clinical characteristics of the study participants.

Characteristics		Patient Group N=60	Control Group N=30
Demographic	**Age** (40-50 year) (51-61 year) (≥62 year)	 7 (11.7 %) 29 (48.3%) 24 (40%)	 14 (46.7%) 9 (30%) 7 (23.3%)
	Sex (male/female)	(30/30) (50%/50%)	(15/15) (50%/50%)
	BMI Normal Overweight Obese	 17 (28.3%) 20 (33.3%) 23 (38.3%)	 15 (50%) 8 (26.7%) 7 (23.3%)
Medical History	FH Premature CAD (Yes/ No)	(13 /47) (21.7%/78.3)	(6/24) (20%/80%)
	Smoking (Yes/No)	(12/48) (20%/80%)	(4/26) (13.3%/86.7%)
	DM (Yes/No)	(36/24) (60%/40%)	(4/26) (13.3%/86.7%)
	HTN (Yes/No)	(44/16) (73.3% /26.7%)	(12/18) (40% /60%)
	Physical activity(Yes /No)	(36 /24) (60% /40%)	(30/0) 100%
	KnownCAD (Yes/No)	(37/23) (61.7% /38.3)	–
	ACE- inhibitors Drug(yes/no)	(45/15) (75.0% /25.0%)	–
	B-Blocker Drug(yes/no)	(40/20) (66.7% / 33.3%)	–

BMI: Body mass index, DM: Diabetes mellitus, HTN: Hypertension, FH Premature CAD: family history of premature Coronary artery disease, KnownCAD: Known Coronary artery disease, ACE- inhibitors :Angiotensin –converting enzyme inhibitors, B-Blocker: Beta -Blocker

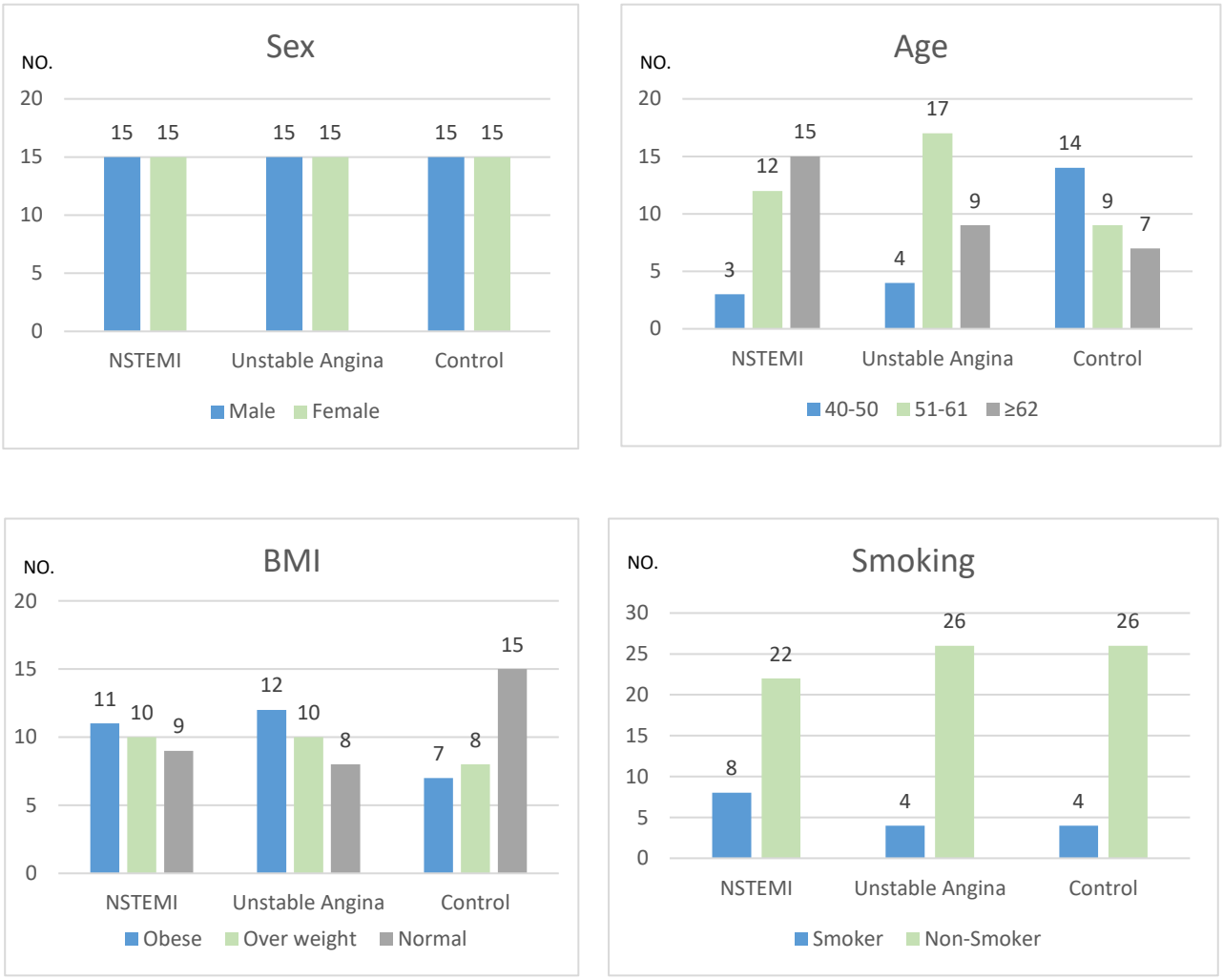


Figure (3-1): Distribution of patients and Control group according to sex, age group, BMI, and smoking status.

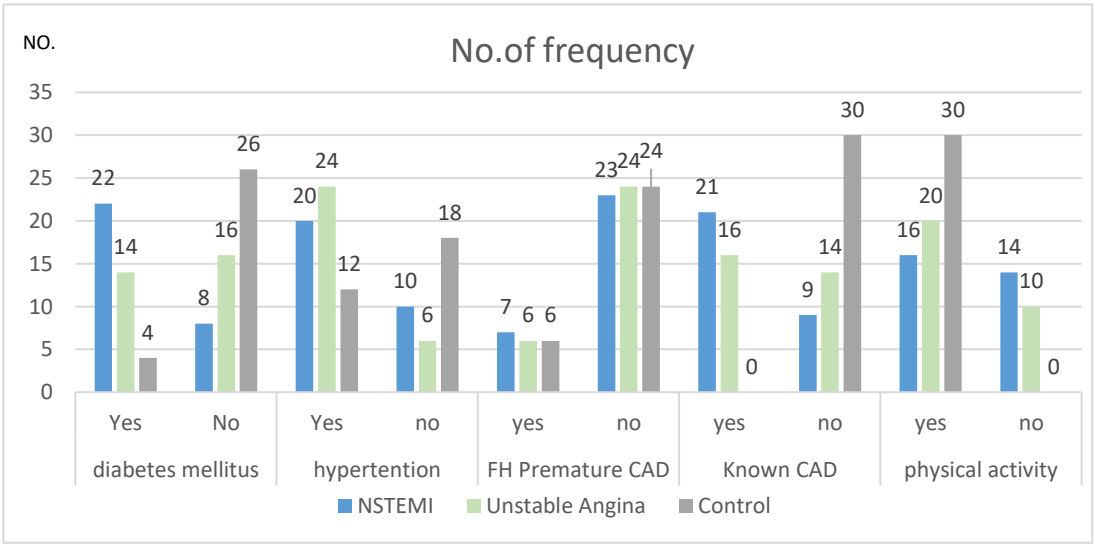


Figure (3-2): Distribution of patients and Control group according to Medical history of participants.

3.2. determination of the serum levels of sST2 and IGFBP2 in NSTE-ACS group compared to control group

In general, patients with NSTE-ACS showed a significantly decreased serum level of sST2 ($p \leq 0.000$) compared to the control group, the median (Min-Max), IQR were 654.0 (202.3-1576.3), 361.72 and 986.35(451.6-2016.3), 554.36 respectively. On the other hand, the difference in the serum level IGFBP2 between the patient group and the control group was not statistically significant ($p=0.560$), the median (Min-Max), IQR were 124.5 (21.4- 728.8), 260.0 and 124.3 (38.0- 578.3), 250.9 respectively. as shown in table (3-2).

Table (3-2): determination of the serum levels of sST2 and IGFBP2 in NSTE-ACS group compared to control group.

Marker	Patient N=60		Control N=30		P value
	Median (Min-Max)	IQR	Median (Min-Max)	IQR	
sST2 (ng/L)	654.0 (202.3-1576.3)	361.7	986.3 (451.6-2016.3)	554.3	0.000
IGFBP2 (ng/ml)	124.5 (21.4- 728.8)	260.0	124.3 (38.0- 578.3)	250.9	0.560

Mann-Whitney Test was, significant at $p \leq 0.05$; N: number; IQR: interquartile range, Min: Minimum, Max: Maximum

3.3. determination of sST2 and IGFBP2 serum levels for NSTEMI and UA Patients Groups Compared to the Control Group.

When compared the serum levels of sST2 among the patient subgroups of NSTE-ACS, namely NSTEMI and UA with the control group, was observed statistically significant difference ($p \leq 0.000$), the median (Min-Max), IQR were 600.5 (202.3-1576.3) 372.2, 671.6(228.3-1497.7) 373.5 and 986.3 (451.6-2016.3) 554.3 respectively.

On the other hand, a statistically significant difference in IGFBP2 serum levels was observed when compared the subgroups of NSTE-ACS, namely NSTEMI and UA with the control group ($p=0.003$), the median (Min-Max),

IQR were 72.1(24.5-507.9)92.2 ,181.0 (21.4-728.8) 323.1 and 124.3(38.0-578.3) 250.9 respectively .as shown in table (3-3) and figure (3-3).

Table (3-3): determination of sST2 and IGFBP2 serum levels for NSTEMI and UA Patients Groups Compared to the Control Group.

Marker	NSTEMI N=30		Unstable Angina N=30		Control N=30		P value
	Median (Min-max)	IQR	Median (Min-max)	IQR	Median (Min-max)	IQR	
sST2 (ng/L)	600.5 b (202.3-1576.3)	372.2	671.6 b (228.3-1497.7)	373.5	986.3 a (451.6-2016.3)	554.3	0.000
IGFBP2 (ng/ml)	72.1 (24.5-507.9)	92.2	181.0 (21.4-728.8)	323.1	124.3 (38.0-578.3)	250.9	0.003

Kruskal-Wallis Test was, significant at $p \leq 0.05$; N: number; IQR: interquartile range, Min: Minimum, Max: Maximum

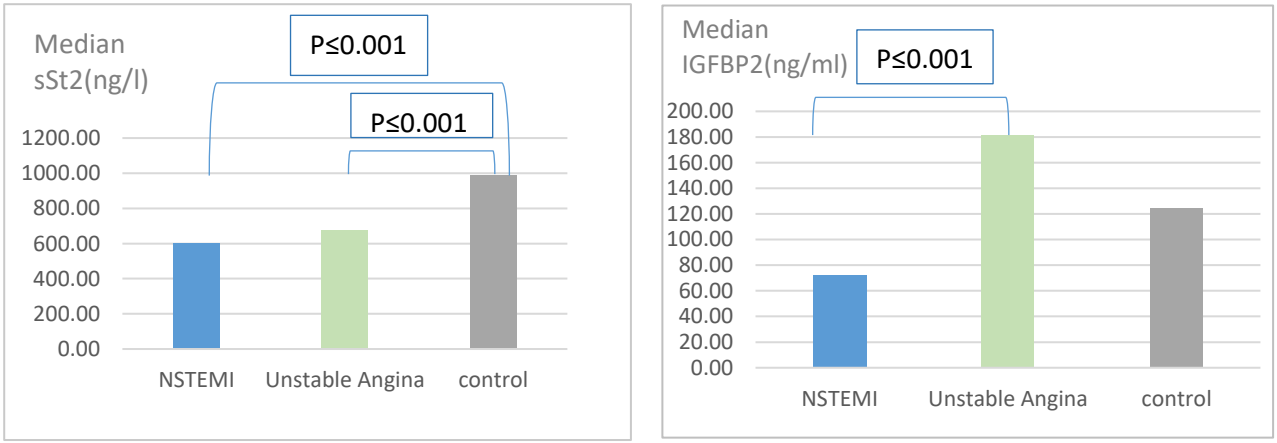


Figure (3-3): The Differences of sST2 and IGFBP2 serum levels, for NSTEMI and UA Patients Groups Compared to the Control Group. (Mann-Whitney Test was, significant at $p \leq 0.05$)

3.4. Comparison of sST2 and IGFBP2 serum levels with heart score in patients with NSTE-ACS.

The study showed no statistically significant difference in the serum levels of sSt2 and IGFBP2 between patients categorized into two risk classes based on the heart score (p=0.336)and (p=0.213). first category consisted of patients with low and medium risk, while the second category included patients at high risk. the median (Min-Max), IQR of sST2 were 642.0 (202.3-1497.7)338.3 and 674.0 (214.1-1576.3) 417.3 respectively. the median (Min-Max), IQR of IGFBP2 were 139.4(21.4-728.8)272.7 and 75.8 (24.5-507.9)200.7 respectively. as shown in table (3-4).

Table (3-4): The Comparison of sST2 and IGFBP2 serum levels with heart score in patients with NSTE-ACS.

Heart score					
Marker	**Low and Medium N=34**		**High N=26**		**P value**
	Median (Min-Max)	**IQR**	**Median (Min-Max)**	**IQR**	
sST2 (ng/L)	642.0 (202.3-1497.7)	338.3	674.0 (214.1-1576.3)	417.3	0.336
IGFBP2 (ng/ml)	139.4 (21.4-728.8)	272.7	75.8 (24.5-507.9)	200.7	0. 213

Mann-Whitney Test was, significant at $p \leq 0.05$; N: number; IQR: interquartile range, Min: Minimum, Max: Maximum

3.5. Comparison of sST2 and IGFBP2 serum levels with TIMI score in patients with NSTE-ACS.

The study found that there was no significant difference in sST2 and IGFBP2 serum levels among patients categorized into three risk strata, low-risk, medium-risk, and high-risk group based on the TIMI score. the median (Min-Max), IQR of sST2 were642.0(228.3- 1555.5) 619.6 , 614.4(202.3-1497.7)373.6 and 662.9(446.5-1576.3)276.4 respectively. the median (Min-Max), IQR of IGFBP2 were

184.1(21.4- 728.8)332.2, 102.6(45.1- 606.0)256.2 and 111.0(24.5- 537.1)110.8 respectively. as shown in table (3-5).

Table (3-5): The Comparison of sST2 and IGFBP2 serum levels with TIMI score in patients with NSTE-ACS.

TIMI Score							
Marker	Low N=18		Medium N=24		High N=18		P value
	Median (Min-Max)	IQR	Median (Min-Max)	IQR	Median (Min-Max)	IQR	
sST2 (ng/L)	642.0 (228.3- 1555.5)	619.6	614.4 (202.3-1497.7)	373.6	662.9 (446.5- 1576.3)	276.4	0.856
IGFBP2 (ng/ml)	184.1 (21.4- 728.8)	332.2	102.6 (45.1- 606.0)	256.2	111.0 (24.5- 537.1)	110.8	0.507

Kruskal-Wallis Test was, significant at $p \leq 0.05$; N: number; IQR: interquartile range, Min: Minimum, Max: Maximum

3.6. Comparison of sST2 and IGFBP2 serum levels with Reynolds score in patients with NSTE-ACS.

The study found that there was no significant difference in sST2 and IGFBP2 serum levels among patients categorized into three risk strata: low-risk, medium-risk, and high-risk group based on the Reynolds score. the median (Min-Max), IQR of sST2 were 702.1(202.3-1576.3)514.2, 679.8(428.3-1555.5)639.0 and 558.4(228.3-922.2)252.4 respectively, the median (Min-Max), IQR of IGFBP2 were 141.8(21.4-728.8)383.8, 106.0(32.4-537.1) 112.0 and 121.9(35.1-606.0)175.5 respectively. as shown in table (3-6).

Table (3-6): The Comparison of sST2 and IGFBP2 serum level with Reynolds score in patients with NSTE-ACS.

Marker	Reynolds Score						
	Low N=27		Medium N=15		High N=18		P value
	Median (Min-Max)	IQR	Median (Min-Max)	IQR	Median (Min-Max)	IQR	
sST2 (ng/L)	702.1 (202.3-1576.3)	514.2	679.8 (428.3-1555.5)	639.0	558.4 (228.3-922.2)	252.4	0.252
IGFBP2 (ng/ml)	141.8 (21.4-728.8)	383.8	106.0 (32.4-537.1)	112.0	121.9 (35.1-606.0)	175.5	0.750

Kruskal-Wallis Test was, significant at $p \leq 0.05$; N: number; IQR: interquartile range, Min: Minimum, Max: Maximum

3.7. Differences of the sST2 and IGFBP2 serum levels between age categories in patient with NSTE-ACS.

The study results showed that there was no statistically significant differences in sST2 and IGFBP2 serum levels between individuals with NSTE-ACS aged 44-59 and those aged 60 or above (P=0.657) and (P=0.455). the median (Min-Max), IQR of sST2 were 662.1 (202.3-1576.3) 420.9 and 602.2 (214.1- 1555.5) 329.0 respectively. the median (Min-Max), IQR of IGFBP2 were150.4 (21.4- 728.8)341.8 and 115.9 (24.5- 606.0)112.4 respectively. as shown in table (3-7).

Table(3-7): Differences of the sST2 and IGFBP2 serum levels between age categories in patient with NSTE-ACS.

Marker	Age (44-59) N=29		Age (≥60) N=31		P value
	Median (Min-Max)	IQR	Median (Min-Max)	IQR	
sST2 (ng/L)	662.1 (202.3-1576.3)	420.9	602.2 (214.1- 1555.5)	329.0	0.657
IGFBP2 (ng/ml)	150.4 (21.4- 728.8)	341.8	115.9 (24.5- 606.0)	112.4	0.455

Mann-Whitney Test was, significant at $p \leq 0.05$; N: number; IQR: interquartile range, Min: Minimum, Max: Maximum

3.8. Differences of the sST2 and IGFBP2 serum levels between male and female in patient with NSTE-ACS.

The study results showed that there was no statistically significant difference in sST2 and IGFBP2 serum levels between male and female patients with NSTE-ACS (P=0.069) and (p=0.359) respectively. The median (Min-Max), IQR of sST2 were 589.9 (202.3- 1248.7)247. and732.3(214.1- 1576.3)710.5 respectively. the median (Min-Max), IQR of IGFBP2 were 105.0(21.4- 606.0)175.6 and135.7(24.5-728.8)311.9 respectively**.** as shown in table (3-8).

Table (3-8): Differences of the sST2 and IGFBP2 serum levels between male and female in patient with NSTE-ACS.

Marker	Male N=30		Female N=30		P value
	Median (Min-Max)	IQR	Median (Min-Max)	IQR	
sST2 (ng/L)	589.9 (202.3- 1248.7)	247.2	732.3 (214.1- 1576.3)	710.5	0.069
IGFBP2 (ng/ml)	105.0 (21.4- 606.0)	175.6	135.7 (24.5- 728.8)	311.9	0.359

Mann-Whitney Test was, significant at p ≤ 0.05; N: number; IQR: interquartile range, Min: Minimum, Max: Maximum

3.9. Differences of the sST2 and IGFBP2 serum levels between BMI categories in patient with NSTE-ACS.

The study findings indicated that there was no statistically significant difference in sST2 serum levels (p=0.267) among different BMI categories (normal, overweight and obese) in patient with NSTE-ACS. the median (Min-Max), IQR of sST2 were 702.14 (375.1-1576.3)464.1, 551.7 (202.3-1200.54)311.8 and 598.8 (214.1-1555.5)464.9 respectively.

Conversely, there was a statistically significant difference in IGFBP2 serum levels observed in the obese BMI group (p≤ 0.000) compared to overweight BMI group

and normal BMI group in patient with NSTE-ACS. the median (Min-Max), IQR were 365.89 (73.1- 728.8) 339.0,102.64 (21.4- 445.5)101.8 and 66.30 (24.5-141.8)59.5 respectively. as shown in table (3-9).

Table (3-9): Differences of the sST2 and IGFBP2 serum levels between BMI categories in patient with NSTE-ACS.

Marker	Normal N=17		Overweight N=20		Obese N=23		P value
	Median (Min-Max)	IQR	Median (Min-Max)	IQR	Median (Min-Max)	IQR	
sST2 (ng/L)	702.14 (375.1-1576.3)	464.1	551.7 (202.3-1200.54)	311.8	598.8 (214.1-1555.5)	464.9	0.267
IGFBP2 (ng/ml)	365.89 (73.1- 728.8)	339.0	102.64 (21.4- 445.5)	101.8	66.30 (24.5- 141.8)	59.5	0.000

Kruskal-Wallis Test was, significant at $p \leq 0.05$; N: number; IQR: interquartile range, Min: Minimum, Max: Maximum

3.10. Differences of the sST2 and IGFBP2 serum levels between Diabetic and non-Diabetic in patient with NSTE-ACS.

The study findings indicated that there was no statistically significant difference in sST2 serum levels (p=0.952) among Diabetic and non-Diabetic in patient with NSTE-ACS. the median(Min-Max), IQR of sST2 were 650.9(202.3-1555.5)374.4 and 654.0(214.1- 1576.3)214.1 respectively.

Conversely, there was a statistically significant difference in IGFBP2 levels(p=0.036) among Diabetic and non-Diabetic in patient with NSTE-ACS, the median (Min-Max), IQR of IGFBP2 were 102.6(21.4- 537.1)119.9 and171.2(38.5-728.8)412.7 respectively. as shown in table (3-10).

Table (3.10) Differences of the sST2 and IGFBP2 serum levels between Diabetic and non-Diabetic in patient with NSTE-ACS.

Marker	Diabetics N=36		Non- Diabetics N=24		P value
	Median (Min-Max)	IQR	Median (Min-Max)	IQR	
sST2 (ng/L)	650.9 (202.3- 1555.5)	374.4	654.0 (214.1- 1576.3)	214.1	0.952
IGFBP2 (ng/ml)	102.6 (21.4- 537.1)	119.9	171.2 (38.5- 728.8)	412.7	0.036

Mann-Whitney Test was, significant at $p \leq 0.05$; N: number; IQR: interquartile range, Min: Minimum, Max: Maximum.

3.11. Differences of the sST2 and IGFBP2 serum levels between Hypertension and non-Hypertension in patient with NSTE-ACS.

The study results showed a statistically significant difference in sST2 serum levels (p=0.024) between patients with NSTE-ACS who had hypertension and those who did not have hypertension. the median(Min-Max), IQR of sST2 were 573.9 (202.3-1555.5)330.2 and 702.1 (447.3-1576.3)600.8 respectively.

in contrast there was no statistically significant difference in IGFBP2 serum levels (p=0. 757) between patients with hypertension and those without hypertension who had NSTE-ACS. the median(Min-Max), IQR of IGFBP2 were 131.7 (21.4-606.0) 188.8 and 88.8(48.7-728.8)439.6 respectively. as shown in table (3-11).

Table (3-11) Differences of the sST2 and IGFBP2 serum levels between Hypertension and non-Hypertension in patient with NSTE-ACS.

Marker	hypertension N=44		Non _hypertension N=16		P value
	Median (Min-Max)	IQR	Median (Min-Max)	IQR	
sST2 (ng/L)	573.9 (202.3-1555.5)	330.2	702.1 (447.3-1576.3)	600.8	0.042
IGFBP2 (ng/ml)	131.7 (21.4-606.0)	188.8	88.8 (48.7-728.8)	439.6	0.757

Mann-Whitney Test was, significant at $p \leq 0.05$; N: number; IQR: interquartile range, Min: Minimum, Max: Maximum

3.12. Differences of the sST2 and IGFBP2 serum levels between Positive ECG and Negative ECG in patient with NSTE-ACS.

The study findings indicated that there was no statistically significant difference in sST2 and IGFBP2 serum levels (p=0.560) (p=0.743) respectively. between patients with positive and negative ECG findings in NSTE-ACS**.** the median(Min-Max), IQR of sST2 were 663.3 (202.3-1576.3)364.1and 646.0 (214.1-1248.7) 299.9 respectively.

the median (Min-Max), IQR of IGFBP2 were 127.9 (21.4 - 728.8)281.4 and 119.1(32.4 - 606.0) 207.7 respectively. as shown in table (3-12).

Table (3-12) Differences of the sST2 and IGFBP2 levels between Positive ECG and Negative ECG in patient with NSTE-ACS.

EKG change					
Marker	Positive ECG N=43		Negative ECG N=17		P value
	Median (Min-Max)	IQR	Median (Min-Max)	IQR	
sST2 (ng/L)	663.3 (202.3-1576.3)	364.1	646.0 (214.1- 1248.7)	299.9	0.560
IGFBP2 (ng/ml)	127.9 (21.4 - 728.8)	281.4	119.1 (32.4 - 606.0)	207.7	0.743

Mann-Whitney Test was, significant at p ≤ 0.05; N: number; IQR: interquartile range, Min: Minimum, Max: Maximum

3.13. Differences of the Cardiac marker serum levels between NSTEMI ,UA and control group.

When compared the levels of pro-BNP among the patient subgroups of NSTE-ACS, namely NSTEMI and UA with control group, was found statistically significant difference (p≤0.000), the median (Min-Max), IQR were 533.7 (35.7-1399.9)408.0, 557.9 (213.6-3570.4) 293.5and 890.8 (210.0- 1717.1) 418.6 respectively.

In addition, was observed a statistically significant difference (p≤0.000) in the levels of Creatinine when compared the patient subgroups of NSTE-ACS, namely NSTEMI and UA with the control group, the median (Min-Max), IQR were 0.5 (0.1-1.2)0.2, 0.4 (0.1- 0.9) 0.2 and 0.3 (0.2 - 0.5)0.1 respectively. as shown in table (3-13) and figure (3-4).

Table (3-13): Differences of the Cardiac marker serum levels between NSTEMI, UA and control group.

Marker	NSTEMI N=30		Unstable Angina N=30		Control N=30		P value
	Median (Min-mix)	IQR	Median (Min-mix)	IQR	Median (Min-mix)	IQR	
Pro-BNP (ng/L)	533.7 (35.7-1399.9)	408.0	557.9 (213.6-3570.4)	293.5	890.8 (210.0- 1717.1)	418.6	0.000
Creatinine (mg/dl)	0.5 (0.1- 1.2)	0.2	0.4 (0.1- 0.9)	0.2	0.3 (0.2 - 0.5)	0.1	0.000

Kruskal-Wallis Test was, significant at p ≤ 0.05; N: number; IQR: interquartile range, Min: Minimum, Max: Maximum

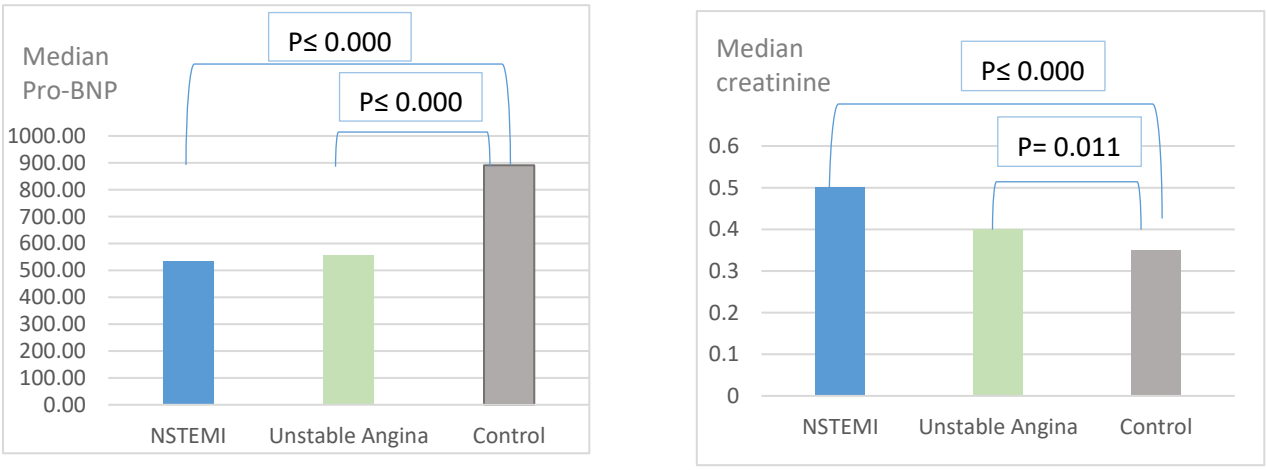


Figure (3-4): The differences of the Cardiac marker serum levels between NSTEMI , UA and control group. (Mann-Whitney Test was, significant at p ≤ 0.05)

3.14. The differences of the Lipid profile levels between NSTEMI, UA and control group.

When compared the serum levels of lipid profile among the patient subgroups of NSTE-ACS, namely NSTEMI and UA with the control group. there was no statistically significant difference in total cholesterol levels across the three groups (p=0.772).

on the other hand, The triglyceride levels showed a statistically significant difference (p=0.012).when compared the patient with NSTEMI and UA with the control group,

the median (Min-Max), IQR were182.0 (39- 392)104, 111 (47- 330)80 and 110 (51-297) 94 respectively.

In addition, we observed a statistically significant difference ($p \leq 0.000$) in the levels of HDL when compared the patient subgroups of NSTE-ACS, NSTEMI and UA with the control group, the median (Min-Max), IQR were 42.50 (22- 111)23,71.0 (29- 96)16 and 71.50 (25- 120) 23 respectively.

When compared the levels of LDL among the patient subgroups of NSTE-ACS which include NSTEMI , UA with control group ,we found statistically significant difference ($p=0.008$) the median (Min-Max), IQR were 90.0 (39- 172)32, 110 (54-220) 39 and 96.0 (54- 159) 28 respectively. as shown in table (3-14) and figure (3-5).

Table (3-14): Differences of the Lipid profile levels between NSTEMI ,UA and control group.

Lipid profile							
Marker	NSTEMI N=30		Unstable Angina N=30		Control N=30		P value
	Median (Min-mix)	IQR	Median (Min-mix)	IQR	Median (Min-mix)	IQR	
Total Cholesterol (mg/dl)	209.5 (121- 426)	87	209.0 (100- 437)	59	197.0 (100- 301)	50	0.772
Triglycerides (mg/dl)	182.0 (39- 392)	104	111.0 (47- 330)	80	110.0 (51- 297)	94	0.012
HDL-Cholesterol (mg/dl)	42.50 (22- 111)	23	71.0 (29- 96)	16	71.50 (25- 120)	23	0.000
LDL-Cholesterol (mg/dl)	90.0 (39- 172)	32	110.0 (54- 220)	39	96.0 (54- 159)	28	0.008

Kruskal-Wallis Test was, significant at $p \leq 0.05$; N: number; IQR: interquartile range, Min: Minimum, Max: Maximum

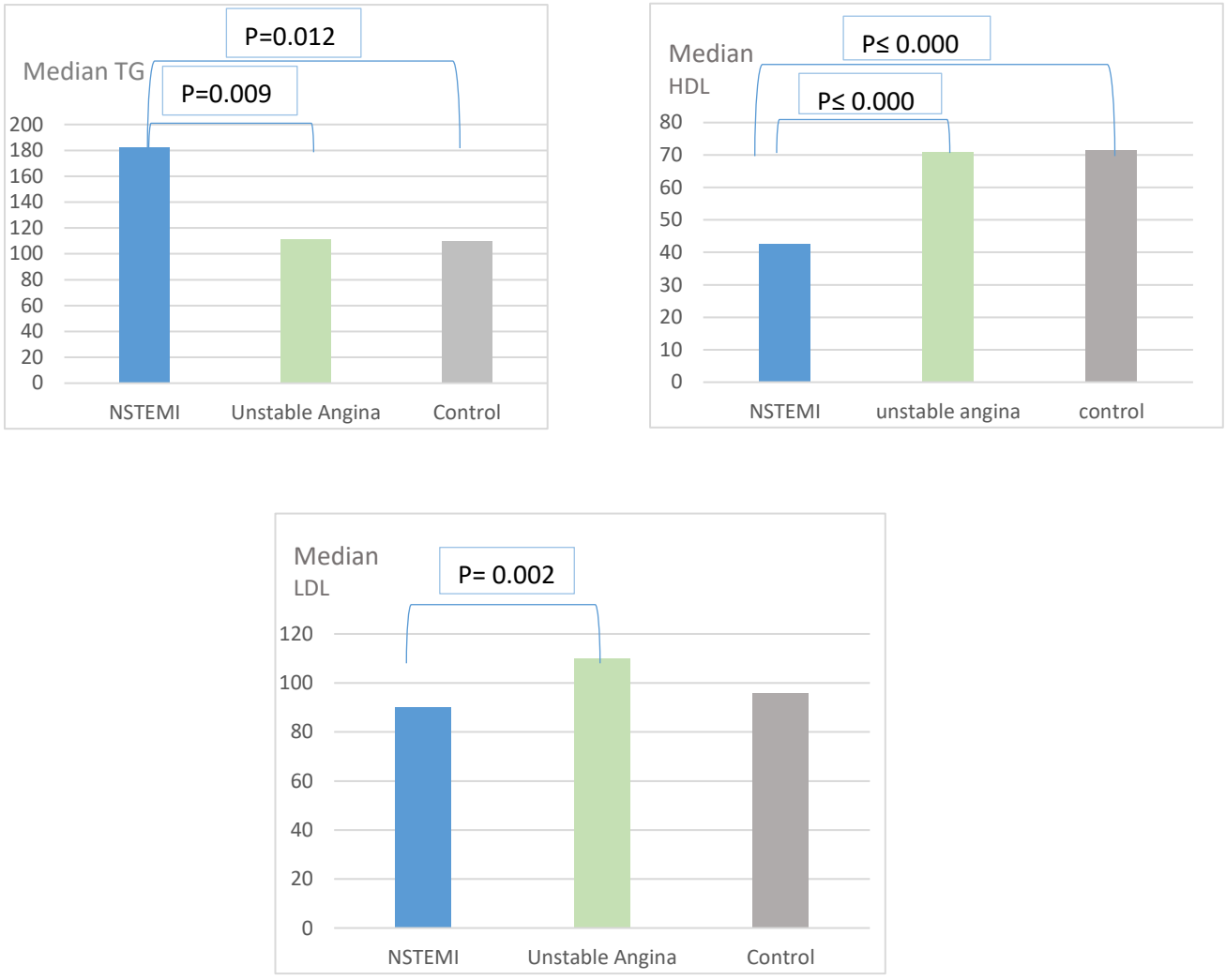


Figure (3-5) Differences of the Lipid profile levels between NSTEMI, Unstable angina and control group. (Mann-Whitney Test was, significant at $p \leq 0.05$)

3.15. Correlations between sST2, IGFBP2 serum level with cardiac risk score in patients with NSTE-ACS.

sST2 demonstrated a significant negative correlation with the Reynolds Score(p=0.048), while there was a positive but non-significant relationship with the Heart Score (p=0.525) and TIMI Score (p=0.829).

On the other hand, IGFBP2 showed a non-significant negative correlation with the Heart Score, Reynolds Score, and TIMI Score (p=0.059), (p=0.251) And (p=0.125) respectively. as shown in table (3-15).

Table (3-15): Correlations between sST2 and IGFBP2 serum levels with cardiac risk score in patients with NSTE-ACS.

Marker	Heart score N=60		Reynolds score N=60		TIMI score N=60	
	r	P value	r	P value	r	P value
sST2 (ng/L)	0.084	0.525	-0.256*	0.048	0.029	0.829
IGFBP2 (ng/ml)	-0.245	0.059	-0.151	0.251	-0.200	0.125

Spearman Rank Test; *Correlation is significant at the 0.05 level; - = negative; r: pearson correlation coefficients

3.16. Association between sST2 and IGFBP2 serum levels with cardiac marker in patients with NSTE-ACS.

The results indicated that sST2 had a non-significant negative correlation with hs-Troponin I and Hs-CRP (p=0.779) and (p=0.865), and non-significant positive correlation with Pro-BNP(p=0.778) .

In contrast, IGFBP2 showed a significant Negative correlation with hs-Troponin I (p≤0.001) and non-significant positive correlation with Pro-BNP (p=0.197). and There was a non-significant negative correlation with HS-CRP (p=0.559). as shown in table (3-16).

Table (3-16): Correlations between sST2 and IGFBP2 serum levels with cardiac marker in patients with NSTE-ACS.

Marker	hs-Troponin I N=60		PRO-BNP N=60		hs-CRP N=60	
	r	Sig.	r	Sig	r	Sig
sST2 (ng/L)	-0.037	0.779	0.037	0.778	-0.022	0.865
IGFBP2 (ng/ml)	-0.407**	0.001	0.169	0.197	-0.077	0.559

Spearman Rank Test; **Correlation is significant at the 0.01 level; *Correlation is significant at the 0.05 level; - = negative; r: Pearson correlation coefficients; Hs-Troponin I: high- sensitivity Troponin I; Hs-CRP: High-sensitivity C-reactive protein; Pro-BNP: pro-Brain Natriuretic peptide

3.17. Correlations between sST2 and IGFBP2 serum levels with lipid profile in patients with NSTE-ACS.

The findings in patients with NSTE-ACS demonstrated a significant positive correlation between sST2 and LDL (p=0.010). Both The Total Cholesterol and HDL levels displayed a non-significant positive correlation with sST2 level (p=0.062) and(p=0.081), whereas TG demonstrated a non-significant negative correlation with sST2 level (p=0.260).

On the other hand, IGFBP2 had a significant positive correlation with both LDL (p=0.025) and HDL(p≤0.000), and a significant negative correlation with TG (p≤0.000).There was a non-significant positive correlation observed with cholesterol levels (p=0.764) . as shown in table (3-17).

Table (3-17): Correlations between sST2 and IGFBP2 serum levels with lipid profile in patients with NSTE-ACS.

Marker	Total Cholesterol N=60		Triglycerides N=60		HDL-Cholesterol N=60		LDL-Cholesterol N=60	
	R	Sig.	r	Sig.	r	Sig.	r	Sig.
sST2 (ng/L)	0.242	0.062	-0.148	0.260	0.227	0.081	0.330*	0.010
IGFBP2 (ng/ml)	0.040	0.764	-0.821**	0.000	0.579**	0.000	0.289**	0.025

Spearman Rank Test; **Correlation is significant at the 0.01 level; *Correlation is significant at the 0.05 level; - = negative; r: pearson correlation coefficients

3.18. Study the association of sST2 with NSTE-ACS patients.

Binary logistic regression was performed to analyze the associating of the sST2 with NSTE-ACS, It was found the sST2 biomarker shown a highly significant differences in patient and represented as a risk factor (OR: 1.002; 95% CI: (1.001-1.004). as shown in table (3-18).

Table (3-18): Estimation the Associated of sST2 serum level in NSTE-ACS Patients Compared to control group.

Marker	OR(Lower–Upper)	P value
sST2 (ng/L)	1.002 (1.001-1.004)	0.000

OR: Odds Ratio, CI; Confidence Interval,

3.19. Receiver operating characteristic (ROC) .

3.19.1. ROC Curve and AUC Analysis for the sST2 for NSTEMI Patients Group Compared to the Control group.

ROC curve and AUC analysis for the sST2 for NSTEMI Patients compared to control group were performed. Results of the receiver operating curve (ROC) curve and AUC analysis for the sST2 diagnostic parameter were shown that sST2 have a fair performance for NSTEMI patients, data are presented in Figure (3-6) and table (3-19), sST2 had AUC =0.754 [95% Cl (confidence interval) =0.629-0.879], Sensitivity% =0.667, Specificity% = 0.800, Cut-off points = 708.1 (ng/L).

Table (3-19): AUC, optimal threshold, sensitivity, and specificity of sST2 obtained by the ROC curves in patient with NSTEMI compared to Control group.

Test Variable	Cut-off points	Sensitivity	Specificity	AUC	CI (95%)	p-value
sST2	< 708.1 (ng/L)	0.667	0.800	0.754	0.629 -0.879	0.001

ROC: Receiver operating characteristic; significant at p ≤ 0.05 ; AUC; Area under curve,

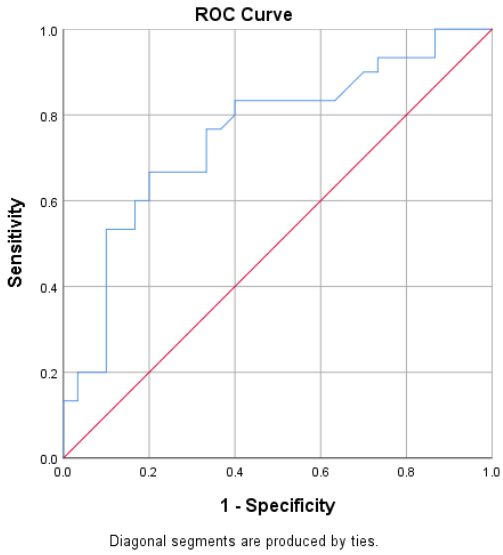


Figure (3-6): Receiver operating characteristic(ROC) of sST2 in NSTEMI patients compared to Control group .

3.19.2. ROC Curve and AUC Analysis for the sST2 for UA Patients Group Compared to the Control group.

ROC curve and AUC analysis for the sST2 for UA Patients compared to control group were performed. Results of the receiver operating curve (ROC) curve and AUC analysis for the sST2 diagnostic parameter were shown that sST2 have a fair performance for UA patients, data are presented in Figure (3-7) and table (3-20), sST2 had AUC =0.753 [95% Cl (confidence interval) =0.631-0.875], Sensitivity% =0.800, Specificity% = 0.667, Cut-off points = 875.7 (ng/L).

Table (3-20): AUC, optimal threshold, sensitivity, and specificity of sST2 obtained by the ROC curves in patient with UA compared to Control group.

Test Variable	Cut-off points	Sensitivity	Specificity	AUC	CI (95%)	p-value
sST2	< 875.73 (ng/L)	0.800	0.667	0.753	0.631-0.875	0.001

ROC: Receiver operating characteristic; significant at p ≤ 0.05 ; AUC; Area under curve,

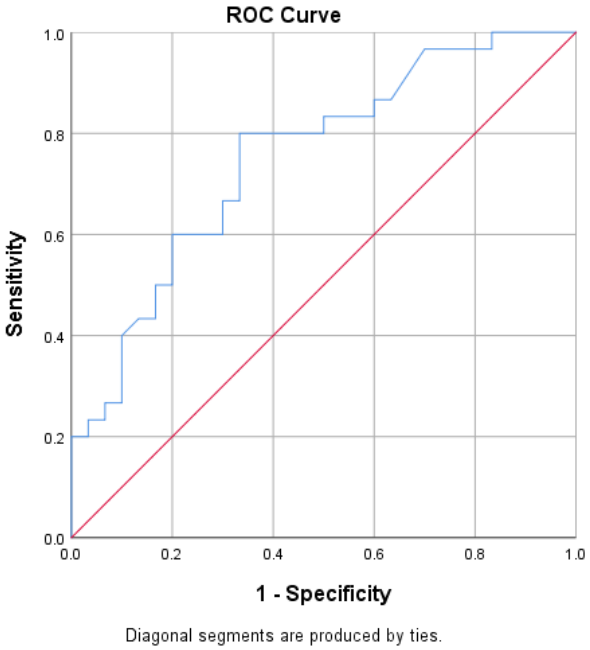


Figure (3-7): Receiver operating characteristic(ROC) of sST2 in UA patients compared to Control group.

3.19.3. ROC Curve and AUC Analysis for the IGFBP2 for UA Patients Group Compared to the NSTEMI patients.

ROC curve and AUC analysis for the IGFBP2 for UA Patients compared to NSTEMI patients. Were performed. Results of the receiver operating curve (ROC) curve and AUC analysis for the IGFBP2 diagnostic parameter were shown that IGFBP2 have a fair performance for UA patients, data are presented in Figure (3-8) and table (3-21), IGFBP2 had AUC =0.743 [95% Cl (confidence interval) =0.612-0.874], Sensitivity% =0.767, Specificity% = 0.700, Cut-off points = 117.5 (ng/L).

Table (3-21): AUC, optimal threshold, sensitivity, and specificity of IGFBP2 obtained by the ROC curves in patient with UA compared to NSTEMI patients.

Test Variable	Cut-off points	Sensitivity	Specificity	AUC	CI (95%)	p-value
IGDBP2	>117.5 (ng/ml)	0.767	0.700	0.743	0.612-0.874	0.001

ROC: Receiver operating characteristic; significant at p ≤ 0.05 ; AUC; Area under curve,

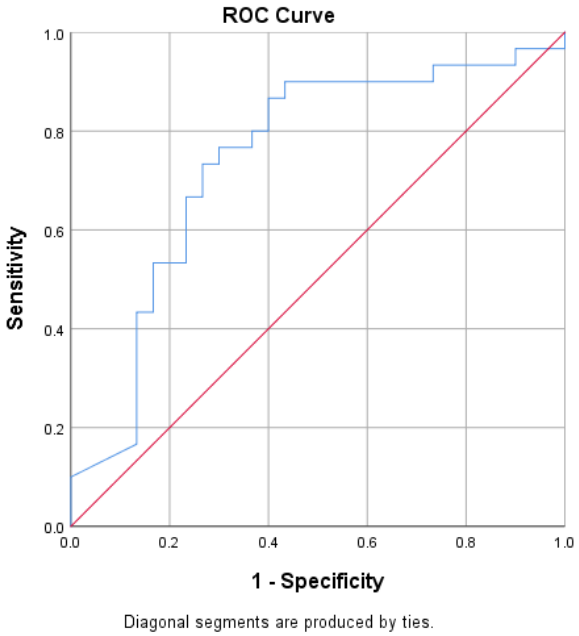


Figure (3-7): Receiver operating characteristic(ROC) of IGFBP2 in UA patients compared to NSTEMI patients.

Chapter Four

Discussion

4. Discussion

4.1. Demographics and Clinical Characteristics

in the current study, the percentage of Male is equal to that of Female. as shown in the Table (1-3).A research by Barton in 2023 showed Cardiovascular medicine, including the management of myocardial infarction (MI), has traditionally been seen as a condition mostly affecting men. Although men have a higher occurrence of myocardial infarction (MI), women tend to have a higher mortality rate when considering age and other known factors that may affect the results (Barton et al., 2023). The cardiovascular risk in postmenopausal women increases significantly as a result of the decline in estrogen levels, which typically provide cardiovascular protection, a greater occurrence of cardiovascular risk factors, and hereditary susceptibility (Kim and Kim, 2023). Sex is a potential risk factor in aging adults, as older ladies have a higher risk of cardiovascular disease (CVD) compared to men of the same age. Nevertheless, in both males and females, the likelihood of developing cardiovascular disease (CVD) rises as they become older, which is linked to a general decrease in sex hormones, particularly estrogen and testosterone (Rodgers et al., 2019).

The current study observed that the majority of patients fell into the age range of 51 to 61 years (48.3%), while the age group of 62 years and above accounted for (40%). A study conducted by Benjamin in 2019 found that elderly adults have a much greater occurrence of cardiovascular disorders compared to other demographic groups (Benjamin et al., 2019).

The Rodgers in 2019 study have previously reported similar finding The aging and old population are highly vulnerable to cardiovascular disease. Age is a standalone risk factor for cardiovascular disease (CVD) in adults, although these risks are amplified by other factors such as frailty, obesity, and diabetes. These factors are recognized to exacerbate and intensify cardiac risk factors that are linked to the beginning of advanced age (Rodgers et al., 2019).

The current study observed that a significant proportion of patients were classified as obese (38.3%) and overweight (33.3%). According to commonly understood Obesity is closely linked to the outlook of atherosclerosis, a defining characteristic of coronary artery disease (CAD) and the primary cause of mortality from cardiovascular disease (CVD). Adipose tissue primarily functions as an organ for storing lipids. It also secretes cytokines called adipokines, which can have significant effects on the formation and progression of atherosclerosis (Liu et al., 2022).

Additionally,in the present study 60% of the patients were diagnosed with type 2 diabetes, whilst 73.3% have hypertension. Type 2 diabetes mellitus (DM) and hypertension (HTN) are widely recognized factors that elevate the likelihood of developing cardiovascular disease (CVD). In addition, individuals who have both diabetes mellitus (DM) and hypertension (HTN) face an increased risk of cardiovascular mortality compared to those who have only one of these illnesses (Al-Azzam et al., 2021).

More than a third Approximately of patients in the present study did not engage in physical activity. Numerous clinical research have demonstrated that physical activity preserving endothelial function, where the Physical exercise has the potential to provide several positive effects in the recovery of endothelial dysfunction. These effects include reducing oxidative stress, increasing the presence of circulating endothelial progenitor cells (EPC), and inhibiting pro-inflammatory cytokines(Gao et al., 2022).

4.2. Soluble suppression of tumorigenicity 2 (sST2).

The findings showed a reduction in the concentration of sST2 in the groups of patients, as indicated in Table (3-2). Multiple studies have been undertaken on the concentrations of sST2 in cardiovascular diseases. The administration of B-blocker medication was found to significantly improve left ventricular function,

leading to a decrease in the size of the infarction and an enhancement of IL-33 / ST2 signaling. As a result, there was a decline in the production of sST2, The study revealed that the administration of β-blocker medicine resulted in a reduction in sST2 levels, which is a fascinating finding.Thus, it was concluded that β-blocker medication can greatly influence the regulation of IL-33 / ST2 signaling and ventricular remodeling(Grakova et al., 2022).ACEIs (angiotensin-converting enzyme inhibitors) medication reduce the synthesis of angiotensin II ,Angiotensin II is a peptide that is generated from angiotensinogen by the enzymatic process of ACE. It has a vital role in inflammatory processes, T-cell response, and cardiac fibrosis. Angiotensin stimulates the synthesis of sST2, which in turn affects the binding of IL-33. ACE inhibitors (ACEIs) limit the secretion of inflammatory cytokines, hence diminishing the impact of Angiotensin II. The decrease in sST2 levels enables IL-33 to bind to ST2L, which offers protection to the heart against cardiac fibrosis (Ambari et al., 2020).

The decline in sSt2 levels seen in the patients of the present study can be ascribed to their current drug intake.

The meta-analysis conducted by Zhang in 2021 revealed that there was no statistically significant disparity in blood sST2 levels between individuals with ischemic heart disease (IHD) or myocardial infarction (MI) and those who were in good health. Nevertheless, patients with HF experienced a significant rise in serum sST2 levels (Zhang et al., 2021). Multiple studies have shown that blood sST2 levels have a tendency to rise after a myocardial infarction (MI), especially over the 3 to 5 day timeframe(Karimzadeh et al., 2017).Given the pathophysiology of sST2 in the cardiovascular disorders mentioned, it is hypothesized that levels of sST2 may rise after the acute phase of myocardial infarction. However, further proof is required to verify this (Zhang et al., 2021).

When the NSTE-ACS patients were divided into two groups, NSTEMI and UA, the current study found no statistically significant difference between them as shown in

the Table (3-3) and figure (3-3). The distribution of sST2 does not have a substantial value for ACS. This aligns with the fact that increases in sST2 levels are not exclusive to acute myocyte cell death, but instead indicate a non-specific inflammatory reaction (Marino et al., 2017).

The current study observed no statistically significant differences in sSt2 levels between age groups, genders and BMI in patients. As stated in Tables(3 -7),(3-8) and (3-9) In accordance with prior researches, the results of Riccardi's study in 2023 Factors such as age, sex, and body mass index do not have a significant influence on the concentration of sST2 (Riccardi et al., 2023). Beetler 2023 is in agreement with this. There were not significant differences in sST2 levels between males and females with coronary artery disease (CAD) in patients aged 50 and above (Beetler et al., 2023) compared to other biomarkers, sST2 has the benefit of being unaffected by confounding factors such as, BMI, or age. Its levels are only altered by the progression of the disease (Farcaş et al., 2020).

In the present investigation, the level of sST2 in patients with NSTE-ACS showed no significant differences in the diabetes group compared to the non-diabetic group, As stated in Table(3-10) .A study conducted by Simeon found no increase in sST2 levels in individuals with (T2DM) (Simeone et al., 2022).Type 2 diabetes is linked to a higher likelihood of developing cardiovascular diseases, including coronary heart disease. Recent research has indicated that persons with prediabetes can exhibit signs of CVD, specifically subclinical atherosclerosis. This indicates that cardiovascular disease (CVD) may occur before or at the same time as the beginning of metabolic disease.(Hasan and Aldhahi, 2020). This explains why no association was found between sST2 and T2D in the current study, and this could be attributed to the possibility that the participants surveyed had recently been disease and were not aware of it. In the diabetes group Li showed a significantly greater level of sST2 compared to the non-diabetic group (Li et al., 2021).

The current study observed reduction in the levels of sST2 in the hypertension group compared to the non-hypertension group ,As stated in Table(3-11) Hypertension is characterised by activation of the sympathetic nervous system from early to late phases, which makes beta-blockers an appropriate treatment (Esler et al., 2022). The study by Xia in 2017 suggested that β-blocker therapy modulates IL-33 / sST2 signaling, thereby slowing down the processes of ventricular remodeling leading to a decrease in sST2 expression (Xia et al., 2017) .ACE inhibitors are used as a first-line medication to be prescribed to treat hypertension, (Le et al., 2021). Renin-angiotensin system inhibitors including ACEIs can reduce sST2 (Mahendra et al.)

The results of the present study indicated a non-significantly negative correlation between sST2 along with Hs-CRP and Hs-troponin as indicated in Table. (3-16) The Katsioupa 2023 study, as well as prior studies, have shown similar findings. They discovered that there was no statistically significant association between sST2, troponin, and ECG. This implies that ST2, when used as a biomarker, indicates specific pathophysiological pathways that are separate from cardiac damage and the burden of ischemia/infarction (Katsioupa et al., 2023)

Zhang observed a significant correlation between elevated levels of sST2 and the inflammatory marker hs-CRP. which suggests the existence of chronic inflammation in the development of atherosclerosis. Studies have shown that beta-blockers can hinder the formation of fibrous tissue, decrease the extent of tissue damage caused by a lack of blood supply, and enhance the functioning of the heart by promoting the IL-33/ST2 signaling pathway. This interaction influences the immune response, restricting the inflammation and progression of plaque (Zhang et al., 2022). The current study showed a strong and direct correlation between sST2 and LDL as indicated in Table.(3-17). These results are consistent with the conclusions of prior study, which also emphasized A positive connection was found between sST2 and LDL cholesterol(Ates et al., 2016).

4.3.Insulin-like growth factors2 (IGFBP2)

The results of the current study showed no statistically significant difference in the concentration of IGFBP2 between patients with NSTE-ACS and the control group, as shown in Table (3-2). A study conducted by Wang discovered that levels of IGFBP-2 were elevated in patients with acute myocardial infarction compared to those in healthy individuals, However, there is a limited amount of research assessing the impact of IGFBP-2 levels on cardiovascular disease. (Wang et al., 2023b). Patients diagnosed with ACS exhibited dramatically reduced serum levels of (IGFBP-2). Concentrations of IGFBP-2 had a negative correlation with intima-media thickness and pulse wave velocity, indicating a negative association with atherosclerosis (Topf et al., 2022, Hoeflich et al., 2018). Research on the role of insulin-like growth factor binding protein-2 (IGFBP-2) in atherosclerosis is scarce(Wang et al., 2024).

The present study did not identify any statistically significant variances in IGFBP2 levels among individuals with NSTE-ACS across the age groups and genders ,As indicated in the tables (3-7)and(3-8).Van Den Beld shown that serum IGFBP-2 levels exhibit a progressive rise with advancing age, namely after reaching the age of 50 years (Van Den Beld et al., 2019). This could be associated with decreased insulin sensitivity and impaired beta-cell activity (Watts et al., 2019). Mester observed that there were no significant differences in plasma IGFBP-2 levels between males and females (Mester et al., 2023). The present investigation found no statistically significant disparities in IGFBP2 levels between the UA/NSTEMI patients with hypertension and those without hypertension as indicated in Table

(3-11) Currently, there is a lack of information on the correlation between IGF-1, its binding proteins, and blood pressure (BP)(Schlueter et al., 2024).

The results of the current study demonstrated a reduction in the concentration of IGFBP2 in diabetic patients compared to the non-diabetic group, and it also

decreased in obese individuals compared to other categories, as indicated in Tables (3-9) and(3-10). This is consistent with prior research findings. The concentration of IGFBP2 was found to be lower in patients with type 2 diabetes compared to non-diabetic persons. The IGF/IGFBP2 system plays a role in the insulin system pathway (Boughanem et al., 2021). High expression of IGFBP2 provides protection against type II diabetes and regulates glucose metabolism (Alicea et al., 2024). IGFBP-2 promotes the absorption of glucose in adipocytes through the GLUT4 pathway, indicating a direct association with insulin signaling pathways (Wittenbecher et al., 2019).

Obesity leads to a decrease in the levels of IGFBP-2 in the bloodstream (Hoeflich and Russo, 2015). Lau 2021 showed lower levels of IGFBP2 are linked to adverse metabolic risk factors, including increased BMI , reduced insulin sensitivity, and less favorable lipid profiles. Recent clinical studies have demonstrated that IGFBP-2 is one of the most effective biomarkers that is inversely correlated with metabolic syndrome(Lau et al., 2021) IGFBP2 is sensitive to relevant changes in an individual's lifestyle. in which increased levels of IGFBP2 are associated with improvements of metabolic variables in obesity and insulin sensitivity (Boughanem et al., 2021).

In current study Plasma IGFBP-2 levels exhibited an significant negative correlation with triglyceride levels and a significant positive correlation with HDL and LDL levels, as shown in the Table (3-17). However, there was no correlation observed between IGFBP-2 and cholesterol levels. The results of current study align with previous research that identified Levels of IGFBP-2 showed a negative correlation with plasma (TG) and a positive correlation with high-density lipoprotein (HDL-cholesterol). The findings suggest that the connection could be attributed to a combination of decreased elimination of VLDL and IDL particles having apoB-100, as well as increased production of chylomicrons with apoB-48. The negative correlation between circulating IGFBP-2 and TG levels is probably

influenced by these two putative kinetic variables (Rauzier et al., 2022). WANG in 2023 noted that high IGFBP-2 group patients had lower triglyceride levels and BMI. It was findings are in agreement with prior literature that reported IGFBP-2 to be a marker of metabolic syndrome and inversely correlate with BMI and triglyceride levels (Wang et al., 2023b).

The findings of the current investigation revealed a non-significant negative link between IGFBP2 and Hs-CRP, but a significant negative correlation was observed with Hs-Troponin as shown in the Table (3-16). The study conducted by Dong indicates a significant correlation between elevated serum levels of IGFBP2 and CRP (Dong et al., 2020).

It is believed that IGFBP2 acts as a protective factor, as higher levels of IGFBP2 may be associated with lower troponin levels during unstable angina attacks. This negative association suggests that individuals with a higher level of IGFBP2 may experience less myocardial injury or stress, leading to decreased troponin release. The correlation between IGFBP2 and troponin in the context of unstable angina is an intriguing area of research.

4.4. Cardiac risk scores

The results of the present study indicate that there were no significant differences in sST2 and IGFBP2 levels among patients with NSTE-ACS categorized into three risk groups: low-risk, medium -risk, and high-risk, as determined by Heart score ,TIMI score and Reynolds score, as shown in the Tables (3-4) (3-5) and (3,6). Wang did a study to assess the relationship between plasma IGFBP-2 and TIMI risk in patients with ACS. The results indicated significant and positive correlation between IGFBP-2 levels and TIMI risk levels. And levels of IGFBP-2 have the potential to serve as strong biomarkers for predicting TIMI risk in patients with ACS(Wang et al., 2023a). The current study was the first to investigated the association between Sst2 levels and TIMI score, Reynolds Score,

and Heart Score, as well as the relationship between IGFBP2 levels and Reynolds Score and Heart-Score in patients with NSTE-ACS. Due to the unique approach employed in the current study, it has been challenging to make comparisons or reference past studies.

4.5. Pro-BNP, creatinine and lipid profile

The current study observed a reduction in the levels of pro-BNP in patients with NSTEMI and UA compared to the control group, as indicated in Table (3-13) and figure (3-4). cardiac natriuretic peptides are one of the best biomarkers for predicting outcomes in ACS patients and, moreover, should be determined routinely in all ACS patients (Cordero et al., 2021). Multiple clinical studies have shown that the manipulation of neurohormones in the renin-angiotensin-aldosterone system (RAAS) decreases NT-proBNP levels and leads to positive results. The inhibition of the renin-angiotensin-aldosterone system (RAAS) is accomplished through the administration of pharmacological agents, such as angiotensin-converting enzyme (ACE) inhibitors. ACE inhibitors decrease intravascular volume and heart filling pressure, hence preventing long-term remodeling and reducing levels of NT-proBNP (Hartoto et al., 2016).

The current study showed elevated levels of creatinine in patients diagnosed with non-ST elevation myocardial infarction (NSTEMI) and unstable angina (UA) in comparison to the control group, as indicated in Table (3-13) and figure (3-4).

ACS patients exhibited markedly increased serum creatinine levels compared to the control individuals. Multiple prior investigations have documented similar findings. making it a useful supplementary diagnostic marker. In the prognosis of ACS patients, the measurement of Creatinine (Cr) serves as a crucial tool for identifying individuals who are at a higher risk. Elevated levels could be either a cause or a consequence of ACS (Adam et al., 2018). For patients with acute coronary syndrome (ACS), the daily measurement of serum creatinine levels and the patterns

of change in these levels are highly indicative of the likelihood of experiencing complications and mortality while in the hospital (Al-Beltagy et al., 2021).

In the current study, various abnormalities of the lipid profile were discovered. Regarding total cholesterol levels, there had been no significant differences between NSTEMI patients, UA patients and control group. NSTEMI patients had elevated triglyceride levels in comparison to both UA patients and the control group. in addition to that, a markedly reduced HDL and LDL value was noted in NSTEMI patients in contrast to UA patients, as indicated in Table (3-14) and figure (3-5)

Individuals with ACS have a significant risk due to dyslipidemia, which is characterized by elevated TG levels and reduced HDL-C levels ,Rahman (2021) found that patients diagnosed NSTEMI had the highest occurrence of low levels of HDL-C and high levels of TG. Furthermore, a greater percentage of patients diagnosed with NSTEMI showed increased levels of (TG) compared to those with UA (Rahman et al., 2021). Dyslipidemia is a significant indicator of the seriousness of acute coronary syndrome(Hussein et al., 2023).

it is generally understood that patients NSTEMI may have a higher degree of dyslipidemia compared to patients with UA ,as NSTEMI is a more severe condition and is often associated with a greater burden on the cardiovascular system.

Chapter Five

Conclusion and Recommendation

5. Conclusion and Recommendations

5.1. Conclusion

- The levels of sST2 were significantly decreased in the patients compared to the control group, possibly due to the effects of the B-Blocker and ACE inhibitor medications that the all patients were receiving, It was found that they greatly boosted the function of the left ventricle and improved the signaling of IL-33/ST2, resulting in a notable reduction in sST2 levels. On the other hand, there were no statistically significant differences observed in IGFBP2 levels between the patients with (NSTE-ACS) and controls.

- The study found no significant differences in the levels of sST2 and IGFBP2 among the three risk categories defined by TIMI Score, Reynolds Score, and Heart Score in patients with non-ST segment elevation acute coronary syndrome (NSTE-ACS). consequently, it was not feasible to categorize patients into Low, moderate, and high-risk groups based on these biomarkers.

5.2. Recommendation

- The current study that was being conducted was found to be novel and had not been mentioned or referenced in any earlier research. Conducting additional research utilizing a larger number of samples and assessment of this novel method in the future was deemed to be of utmost importance.

- It is recommended that an assessment of sST2 levels in individuals with cardiovascular problems before starting ACE inhibitor and B-blocker treatments. whereas Regularly monitoring sST2 levels throughout treatment is crucial for evaluating the patient's response to ACE inhibitors and B-blockers. Fluctuations in sST2 levels can serve as an indicator of the treatment's efficacy in treating cardiovascular problems.

- Further studies can be conducted on ST-elevation myocardial infarction (STEMI) and chronic coronary syndrome to establish the relationship between these markers and the cardiac risk scores.

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Appendices

Questionnaire

For patients with Unstable Angina \ non-STEMI

Sample No:

Patient Data

Age: ☐

Sex: Male female

Smoking: Yes No

SBP: ☐ mmHg DBP: ☐ mmHg

FH Premature CHD Yes No

Physical activity Yes No

History:

≥ 3 RF for CAD:

diabetes mellitus hypertension

hyperlipidemia smoking family history

Known CAD Yes No

Aspirin use (in past 7 days) Yes No

History (ACS) None Slightly Moderately Highly

ECG finding:

Severe angina Yes No

ST deviation Yes No

+Cardiac marker Yes No

EKG Change normal nonspecific St depression

Lab test:

(sST2) Test

IGFBP-2 Test

Pro BNP

Troponin

creatinine

Lipid Profile:

Total Cholesterol

HDL-Cholesterol

Triglycerides

BMI:

Drug:

lipids lowering drug	Yes	No	B-Blocker	Yes	No
Diuretics	Yes	No	ACE- inhibitors	Yes	No
Digoxin	Yes	No			
Nitroglycerin	Yes	No			

وزارة التعليم العالي و
البحث العلمي

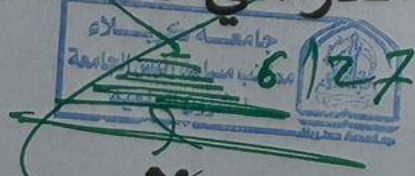
جامعة كربلاء

شهادة استيفاء

نؤيد استيفاء الطالب

زينب هاشم علي حسين

الوحدات الدراسية المطلوبة في نظام تطوير
المهارات الاكاديمية لطلبة الدراسات العليا
للعام الدراسي 2023-2024



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الخلاصة

يندرج كل من احتشاء عضلة القلب بدون ارتفاع ST (NSTEMI) والذبحة الصدرية الغير مستقرة (UA) ضمن فئة متلازمة الشريان التاجي الحادة بدون ارتفاع ST (NSTE-ACS). يتم تمييز NSTEMI عن UA بوجود نخر عضلة القلب الحاد. ويعد إجراء التقييم الفوري للمخاطر أمرًا بالغ الأهمية لتحديد ما إذا كان الأشخاص الذين يعانون من UA / NSTEMI يحتاجون إلى تدخل جراحي مبكر. يعد مؤشر رينولدز ومؤشر القلب ومؤشر TIMI أدوات إنذار تستخدم لتقييم مخاطر الأحداث القلبية الوعائية. بناء على عدة متغيرات. يوجد نوعين من قمع الاورام 2 (ST2) الذي ينتمي إلى عائلة مستقبلات إلانترلوكين _1 (IL-1) وهي: قمع الأورام 2 المرتبط ST2L وهو مستقبل مرتبط بغشاء الخلية و قمع الأورام القابل للذوبان 2(sST2) وهو نسخة قابلة للذوبان توجد في مجرى الدم. يقوم البروتين المرتبط بعامل النمو الشبيه بالأنسولين 2 (IGFBP2)أحد مكونات عائلة IGFBP، بتعديل نشاط عوامل النمو الشبيهة بالأنسولين (IGFs)من خلال التفاعل معها في الدورة الدموية وإعاقة ارتباطها بمستقبلات IGF .

تهدف هذه الدراسة إلى فحص العلاقة بين مستويات sST2 وIGFBP-2 مع ومؤشر TIMI ومؤشر رينولدز ومؤشر القلب لدى المرضى الذين يعانون من متلازمة الشريان التاجي الحادة غير مرتفع ST .تم تصنيف المخاطر الى منخفضة ومعتدلة وعالية بناء على المعايير السريرية اذ يمكن ان يساعد هذا التصنيف الأطباء في اتخاذ قرارات اكثر وضوحا فيما يتعلق بخيارات علاج المرضى

هذه الدراسة هي دراسة الحالات والشواهد تشمل من 90 فردا،تتراوح أعمارهم بين 44-70 سنه شملت العينة 30 مريضاً يعانون من احتشاء عضلة القلب بدون ارتفاع ST، و30 مريضاً يعانون من الذبحة الصدرية الغير مستقرة ، و30 فرداً في المجموعة الضابطة. أجريت الدراسة في مركز كربلاء لأمراض وجراحة القلب. وقام الأطباء المتخصصون بتقييم وتشخيص المرضى .تم جمع المشاركين على مدى الفترة الممتدة من أكتوبر 2023 الى يناير 2024. تم اجراء استبيان لجمع معلومات المرضى، بما في ذلك التركيبة السكانية وعوامل الخطر والتاريخ الطبي ونتائج الفحص البدني ومجموعة من فحوصات الدم مثل sST2, IGFBP2 ,البيتيد المدرة للصوديوم ,التروبونين القلبي عالي الحساسية ,اختبار ملف الدهون ,اختبار البروتين المتفاعل C عالي الحساسية و مستويات الكرياتينين.

كان لدى المرضى الذين يعانون من (NSTEACS) انخفاض كبير في تركيز sST2 (p<0.000) مقارنة بالمجموعة الضابطة. في حين لم يكن هناك فروق ذات دلالة إحصائية في مستوى IGFBP2 (p=0.560) بين مجموعة المرضى والمجموعة الضابطة. من ناحية أخرى لم تكن هناك اختلافات كبيرة لوحظت في مستويات sST2 وIGFBP2 بين المرضى الذين صنفوا إلى ثلاث مجموعات حسب مستوى الخطر: منخفضة المخاطر، متوسطة المخاطر، وعالية المخاطر. استند التصنيف إلى مؤشر TIMI ومؤشر رينولدز ومؤشر القلب. أظهرت نتائج منحنى تشغيل المتلقي (ROC) أن sST2 كان له أداء عادل كمعلمة تشخيصية للمرضى الذين يعانون من NSTEMI وUA بالمقارنة مع مجموعة التحكم. بالإضافة إلى ذلك، أظهر مستوى IGFBP2 أداءً عادلاً كمعلمة تشخيصية للمرضى الذين يعانون من UA بالمقارنة مع مرضىNSTEMI .

ختاماً، لقد لوحظ في المرضى الذين يعانون من NSTE-ACS، انخفاض مستويات sST2 بشكل واضح ربما بسبب أدوية B-Blocker ومثبطات الإنزيم المحول للأنجيوتنسين التي تعزز وظيفة البطين الأيسر وإشارات IL-33/ST2 .لم يتم العثور على فروق ذات دلالة إحصائية في مستويات IGFBP2 بين المرضى والضوابط. كما لم تجد الدراسة فروقاً ذات دلالة إحصائية في مستويات sST2 وIGFBP2 عبر فئات المخاطر المختلفة بناءً على مؤشر TIMI، ومؤشر رينولدز، ومؤشر القلب لدى المرضى الذين يعانون من NSTE-ACS، و بناء على ذلك لم يكن من الممكن تصنيف المرضى إلى مجموعات منخفضة ومتوسطة وعالية الخطورة باستخدام هذه المؤشرات .

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة كربلاء - كلية الطب

فرع الكيمياء والكيمياء الحياتية

العلاقة بين التثبيط القابل للذوبان للأورام 2 (sST2) وبروتين ربط عامل النمو الشبيه بالأنسولين 2(IGFBP-2) مع درجة المخاطر القلبية لدى المرضى العراقيين الذين يعانون من الذبحة الصدرية غير المستقرة \ احتشاء عضلة القلب بدون ارتفاع ST

رسالة ماجستير

مقدمة إلى مجلس كلية الطب/ فرع الكيمياء والكيمياء الحياتية / جامعة كربلاء كجزء من متطلبات نيل درجة الماجستير في (الكيمياء السريرية)

من قبل

زينب هاشم علي

بكالوريوس علوم كيمياء/ جامعة كربلاء/ 2015

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