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Evaluation of lipid profile and Some Immunological parameters in pateints with ischemic heart disease in Karbala province

A Thesis

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in Partial Fulfillment of the Requirements for the Master Degree in Biology Science

By

Zainab Mohammed Neama Al- Hussainii

B.Sc. / Biology /college of Science / University of Kerbala (2005)

Supervised by

Assist. Prof. Dr. Afaf khairi Ismael Ibrahim

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was made under my supervision by **(Zainab Mohammed Neama)** at the collage of the Sciences / University of Karbala in partial fulfillment of the requirements for the degree of M.Sc. Of Science in Biology.

Signature:

Name: Dr. Afaf khairi Ismael Ibrahim

Title: Assist. Professor

Date: 12 / 5 / 2024

Head of Biology Department Certificate

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

Signature:

Name: Dr. Muayad Naeem Kareem

Title: Lecturer

Head of Biology Department, Collage of Science

Date: 12 / 5 / 2024

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We certify that we have read this thesis, entitled **" Evaluation of lipid profile and Some Immunological parameters in pateints with ischemic heart disease in Karbala province"**

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Signature:
Name: Dr. Hussein Jassim AL-Harbi
Scientific degree: Professor
Address: University of Babylon / College of Science
Date: / / 2024

(Chairman)

Signature:
Name: Dr. Mudhafar Sami Khazaal
Scientific degree: Assist. Professor
Address: University of Karbala / College of Medicine
Date: / / 2024

(Member)

Signature:
Name: Dr. Aseel Kariem Jabbar
Scientific degree: Assist. Professor
Address: University of Karbala / College of Science
Date: / / 2024

(Member)

Signature:
Name: Dr. Afaf khairi Ismael Ibrahim
Scientific degree: Assist. Professor
Address: University of Kerbala / College of Science
Date: / / 2024

(Member and Supervisor)

Approved for the council of college

Signature:
Name: Dr. Hassan Jameel Jawad Al-Fatlawi
Scientific degree: Professor
Address: Dean of the College of Science /University of Karbala
Date: / / 2024

DEDICATION

To Allah my Lord,

My homeland Iraq, is the symbol of civilization and the country to I proudly belong, despite the depth of its wounds .

To my spiritual inspiration and my reference* Muhammad Muhammad Sadiq AL-Sadur*, may his soul rest in peace.

To my Family

I dedicate this work.

ZAINAB

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Summery

Ischemic heart disease is a condition brought about by the mean impediment of blood transportation to the muscles of the heart, Lipid profile and immunological disturbances are the foremost factors of danger to IHD. The current study was conducted during the period from December 2022 to May 2023; it included collecting blood samples from (30) individuals randomly selected as a controls group with an age range of 40-85 years as well (60) patients suffering from IHD with age range 40-85 years, whom were visiting the hospital of Imam-AL-Hussein-medical city as well as Imam Al-Hassan Al-Mujtaba hospital in Karbala government . Blood samples were collected from all individuals to evaluate the lipid profile that including (Total cholesterol , Triglycerides , High- density lipoprotein , Low- density lipoprotein, Triglycerides / High density lipoprotein ratio) and immunological biomarkers including (Interleukin -6, soluble Interleukin -6 receptor , Tumor necrosis factor-α).

The study showed significant differences between Ischemic heart disease cases and healthy controls. Ischemic heart disease cases had an increased age range, higher body mass index , and elevated blood pressure levels compared to controls. Biochemical analysis showed increased Total cholesterol, Triglycerides, and Low-density lipoprotein, with decreased High- density lipoprotein, in Ischemic heart disease patients. Immunological parameters were also significantly increased ($P \leq 0.05$) in Ischemic heart disease patients.

The current study studied the distribution of the population by age and the finding showed also highest percentage of Ischemic heart disease patients and controls in the ≥60 age group and the lowest in the ≤50 group. It also examined the effect of age on lipid profile and immunological

biomarkers in Ischemic heart disease, the results showed that (Triglycerides) and (Low-density lipoprotein) levels significantly increased ($P \leq 0.05$) with age, with the highest in the older age groups. Nonetheless, the remaining lipid and immunological biomarkers did not differ significantly across age groups.

The study found that sex had a significant effect on Low-density lipoprotein levels in Ischemic heart disease patients, with females showing higher Low- density lipoprotein compared to males. However, sex did not have a significant effect on other lipid biomarkers or on immunological biomarkers in both Ischemic heart disease patients and healthy controls.

The study found significant correlations between various biomarkers. Height positively correlated with weight, while Triglycerides positively correlated with Total cholesterol, High- density lipoprotein, showed an inverse correlation with height and Total cholesterol, Low- density lipoprotein positively correlated with Total cholesterol and Triglycerides, soluble Interleukin -6 receptor positively correlated with Interleukin -6, The Triglycerides / High-density lipoprotein ratio showed a positive correlation trend with soluble Interleukin -6 receptor, The study also identified cut-off values for Interleukin -6, soluble Interleukin -6 receptor, and Tumor necrosis factor-α in predicting disease activity.

Overall, the results highlight the importance of lipid profile biomarkers, especially Triglycerides / High- density lipoprotein, ratio, as well as Interleukin -6 and Tumor necrosis factor-α in the development of Ischemic heart disease in the Iraqi population, Circulating levels of Interleukin -6, soluble Interleukin -6 receptor, and Tumor necrosis factor-α are more accurate predictors of Ischemic heart disease than lipid profile concentrations.

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List of Abbreviations

Abbreviation	Description
ABCA-1	ATP-binding cassette transporter A1
ABCG-1	ATP-binding cassette transporter G1
ABC	ATP binding cassette cholesterol transporters
ACS	Acute coronary syndrome
ADAM10	A Disintegrin and metalloproteinase 10
ADAM17	A Disintegrin And Metalloproteinase 17
AKT pathway	phosphoinositide 3-kinase (PI3K)-protein kinase B pathway
AMI	Acute myocardial infraction
ANOVA	One-way analysis of variance
APoA1	apolipoprotein A1
APoA2	apolipoprotein A2
APoA-I	apolipoprotein A-I
APoB	apolipoprotein B
APoCs	apolipoprotein Cs
APoE	apolipoprotein E
APoD	apolipoprotein D

APoJ	apolipoprotein J
ATP	Adenosine triphosphate
AUC	Area under the curve
AU	Absorbance Units
B cell	B-Lymphocytes
BMI	Body mass index
BP	Blood pressure
CAD	Coronary artery disease
CANTOS	Canakinumabanti-inflammatory thrombosis outcome study
CETP	Cholesterol ester transfer protein
CHD	Coronary heart disease
CPR	cardiopulmonary resuscitation
CLC	Cardiotrophin –like cytokine
CNTF	Ciliary neurotrophic factor
CT-1	Cardiotrophin-1
CRP	C –reactive protein
DAMPs	Damage-associated molecular patterns
DVT	Deep venous thrombosis
ECG	Eelctrocardiography
Fisher's LS method	FISHER Least Significant Difference (LSD) test in Prism Following one-way (or two-way) analysis
FHS	Framingham Heart Study
gp130	Glycoprotein 130
GK	Glycerokinase
GPO	Glycerol-3-phosphate-oxidase
HDL	High density lipoprotein
HMG-coA	Hydroxy methyl glutaryl-Co enzyme A reductase
HT	Hypertension
H_2O_2	hydrogen peroxide
IDL	Intermediate-density lipoprotein
IFN-γ	Interferon-γ
IHD	Ischemic heart disease
IL-1B	Interleukin-1β
IL-6	Interleukin-6
IL-6Rα	Interleukin-6 receptor α

IL-6R MR	Interleukin-6 receptor Mendelian randomization Analysis
INOCA	ischemia with non-obstructive coronary arteries
JAK-SH2	Janus kinase- domain tyrosine phosphatase 2
JAK1-STAT3	Janus kinase-1 - Signal Transducers and Activators of Transcription-3
JAK family	Janus kinases family of four enzymes
k^+	Potassium
K_{ATP}	ATP-sensitive potassium channel
K_v	voltage-gated potassium channel;
LCAT	Lecithin-cholesterol acyl transferase
LDL	Low density lipoprotein
LIF	Leukemia inhibitory factor
LOX-1	oxidized low-density lipoprotein receptor 1
LPL	Lipoprotein lipase
LPS	Lipopolysaccharide
MAPK	Mitogen-activated protein kinase
mRNA	Messenger Ribonucleic acid
miR-491-5p	Micro Ribonucleic -491-5p
MINOCA	myocardial infarction with non-obstructive coronary arteries
NF-κb	nuclear factor-kappa B
Na_v	voltage-gated sodium channel;
NO	nitric oxide
NLR	Nod-like receptor
NLRB3	Nod-like receptor (NLR) protein-3
OSM	Oncostatin M
Ox-LDL	Oxidized low-density lipoprotein
PAF-AH	Platelet-activating factor acetylhdrolase
PAMPs	Pathogen-associated molecular patterns
PCI	Percutaneous coronary intervention
PCSK-9	Proprotein convertase subtilisin/kexin type 9
PON1	Serum paraoxonase
PR	Plaque rupture
PROCAM	Prospective cardiovascular Munster

PTX-3	Pentraxin 3
P13K	Phosphoinsitide 3-kinase
POD	peroxidase
RA	Rheumatoid arthritis
RAAS	renin–angiotensin–aldosterone system
ROC	Receiver operative characteristic curve
ROS	Reactive oxygen species
SARS-COV-2	severe acute respiratory syndrome coronavirus 2
SHP2	domain tyrosine phosphatase 2
sIL-4R	Soluble interleukin -4 receptor
Sgp130	Soluble gp 130
SMCs	Smooth muscle cell
SOCS1	Suppressor of cytokine signaling -1
SOCS3	Suppressor of cytokine signaling -3
SR-A1	Scavenger receptor class A1
SR-B1	Scavenger receptor class B1
STEMI	S T elevation myocardial infraction
STAT	Signal transducers and activators of transcription factor
STAT3	Signal transducers and activators of transcription factor 3
STNF-α	Soluble tumor necrosis factor alpha
TC	Total cholesterol
T cell	Thymic cells
TG	Triglyceride
TGRLs	triglyceride-rich lipoproteins
TNF-α	Tumor necrosis factor alpha
TNFR1	Tumor necrosis factor alpha receptor 1
TNFR2	Tumor necrosis factor alpha receptor 2
tmTNF-α	Transmembrane Tumor necrosis factor alpha
VLDLs	Very low density lipoprotein
VSMC	Vascular smooth muscle cell
WHO	World Health Organization

Chapter One

Introduction and Literature Review

1.1 Introduction

Ischemic heart disease(IHD): is a pathophysiological condition caused by the disproportion between the myocardial oxygen demand and its supply. Nutrition of the myocardium depends on the oxygen capacity of the blood and the amount of coronary flow (Vollmer-Conna *et al.,* 2015). This reduced blood flow can lead to angina, myocardial infarction, or even unexpectedly cardiac death (Riad *et al.,* 2021). Approximately 75% of abrupt cardiac deaths are due to Coronary artery disease and many of these cases take place in asymptomatic Coronary artery disease patients, who are not conscious of having the disease (Hayashi *et al.,* 2015).

Fifty to sixty percent of people who die from sudden cardiac death have acute coronary thrombus at the site of the occlusion; the other cases show stable coronary plaques with more than 75% cross-sectional area luminal narrowing, either with or without healed myocardial infarction or chronic total occlusion, the most frequent cause of coronary thrombus is plaque rupture. Plaque erosion is the second most prevalent cause of thrombosis and in heavily calcified arteries, calcified nodule is the least common cause of coronary thrombosis (Jinnouchi *et al.,* 2020)

Many studies were confirmed the associations of lipid profiles with the risk of major adverse cardiovascular outcomes in patients with heart disease (Zhao *et al.,* 2021). Blood cholesterol level was identified as the first direct link between circulating lipids and cardiac diseases. Increased cholesterol levels are associated with an increased 10-year risk of cardiovascular death from 3.8% to almost 19.6% in men with a pre-existing cardiac disease, patients with atherosclerotic plaques correlate with 45% higher plasma oxidised Low- density lipoprotein(LDL) concentrations as compared with control subjects. In addition, patients with high total LDL particles have a 3.7 times higher risk of coronary artery calcification than those with lower LDL particles (Prado *et al.,* 2011).

Epidemiological studies also correlate the ratios of other lipids with cardiac diseases risk, demonstrating that increased plasma triglycerides (TG) levels are associated with a 14% increase of cardiac diseases risk in men and a 37% increase in cardiac diseases risk in women, respectively, which is attributed to a higher incidence of myocardial infarction, stroke, and total mortality, subjects with a triglycerides /high density lipoprotein (TG/HDL) ratio >3.5 have an unadjusted hazard risk of cardiac diseases mortality (Vega *et al.*, 2014).

The correlation of the triglycerides /high density lipoprotein (TG/HDL) ratio and cardiovascular risk compared with serum HDL levels is more accurate than individual values because it correlates with the negative impact of TGs and the positive impact of HDL on cardiac diseases (Mach *et al.,* 2020).

The aging of the population is indeed a significant predisposing factor for IHD due to changes in lifestyle, increased prevalence of risk factors like hypertension and diabetes, and physiological changes associated with aging (Jankowski et al., 2018). Traditional and non-traditional risk factors both play a role in the development of IHD in male and female (Mehta *et al.,* 2015).

The modifiable risk factor that encompasses the most frequent factors that can be eliminated or minimized by changing lifestyle habits is typically referred to as "modifiable lifestyle factors" or simply "modifiable risk factors" (Wessler and Kirtane, 2013). These factors include: smoking, dietary habits, physical inactivity, stressful situations, obesity, hypertension, hyperglycemia and lipid disorders (Hajar, 2017; Sharif *et al.,* 2020).

Atherosclerosis, the primary cause of coronary artery disease (CAD), leads to severe events like thrombosis, as well as the rupture or erosion of atherosclerotic plaques (Falk, 2006). This condition is

characterized by chronic inflammation, prompting extensive research into potential mediators initiating and sustaining vascular disease (Feng *et al.*, 2022). A growing body of evidence underscores the pivotal role of inflammation in cardiovascular disease (Guzik and Touyz, 2017).

IL-6 family members modulate the immune response and inflammatory activity and they participate in the development of cardiovascular diseases (Su *et al.*, 2021). Interleukin-6, indeed plays a crucial role in the inflammatory response and has two distinct signaling pathways, one of which is the classic signaling pathway, where IL-6 binds to its specific membrane-bound receptor (IL-6R), In contrast to classical signaling, the second signaling mechanism is termed trans-signaling of IL-6 whereas soluble IL-6 receptor (sIL-6R) is present in various bodily fluids (Huang *et al.*, 2006). In circulation, this interaction forms a complex known as the IL-6/sIL-6R complex or Hyper-IL-6, when this complex binds to the membrane-bound glycoprotein 130 receptor, it triggers a signaling pathway similar to the classical signaling pathway of IL-6, this pathway activation is crucial for various cellular responses involved in inflammation, immune regulation, and other physiological processes (Villar-Fincheira *et al.*, 2021).

At present, the occurrence and development of CAD are generally considered as a chronic inflammatory process, tumor necrosis factor-alpha (TNF-α) is indeed recognized as a significant player in the chronic inflammatory processes associated with CAD (Yuepeng *et al.*, 2019). It has been shown that TNF-α can indeed damage endothelial function, TNF-α can enhance the uptake of oxidized low-density lipoprotein (ox-LDL) by macrophages within the arterial wall, this uptake of ox-LDL by macrophages leads to the formation of foam cells, a key step in the development of atherosclerotic plaques (Gonzálvez *et al.*, 2007; Duan *et al.*, 2021)

1-2 Aim of the study

The current study was designed to evaluate the levels of some immunological and biochemical parameters in patients with ischemic heart disease (IHD) through the following objectives:

1-This prospective study aimed to investigate the relationship between The TG/HDL ratio and incidence of IHD.

2- Measurement the serum levels of IL-6, sILR-6 and TNF-alpha ,Body mass index , Hypertension, Family history of IHD, Smoking Status .

3-The correlation between each one of study parameters development of ischemic heart disease.

2. Literature Reviews

2.1 Ischemic Heart Disease

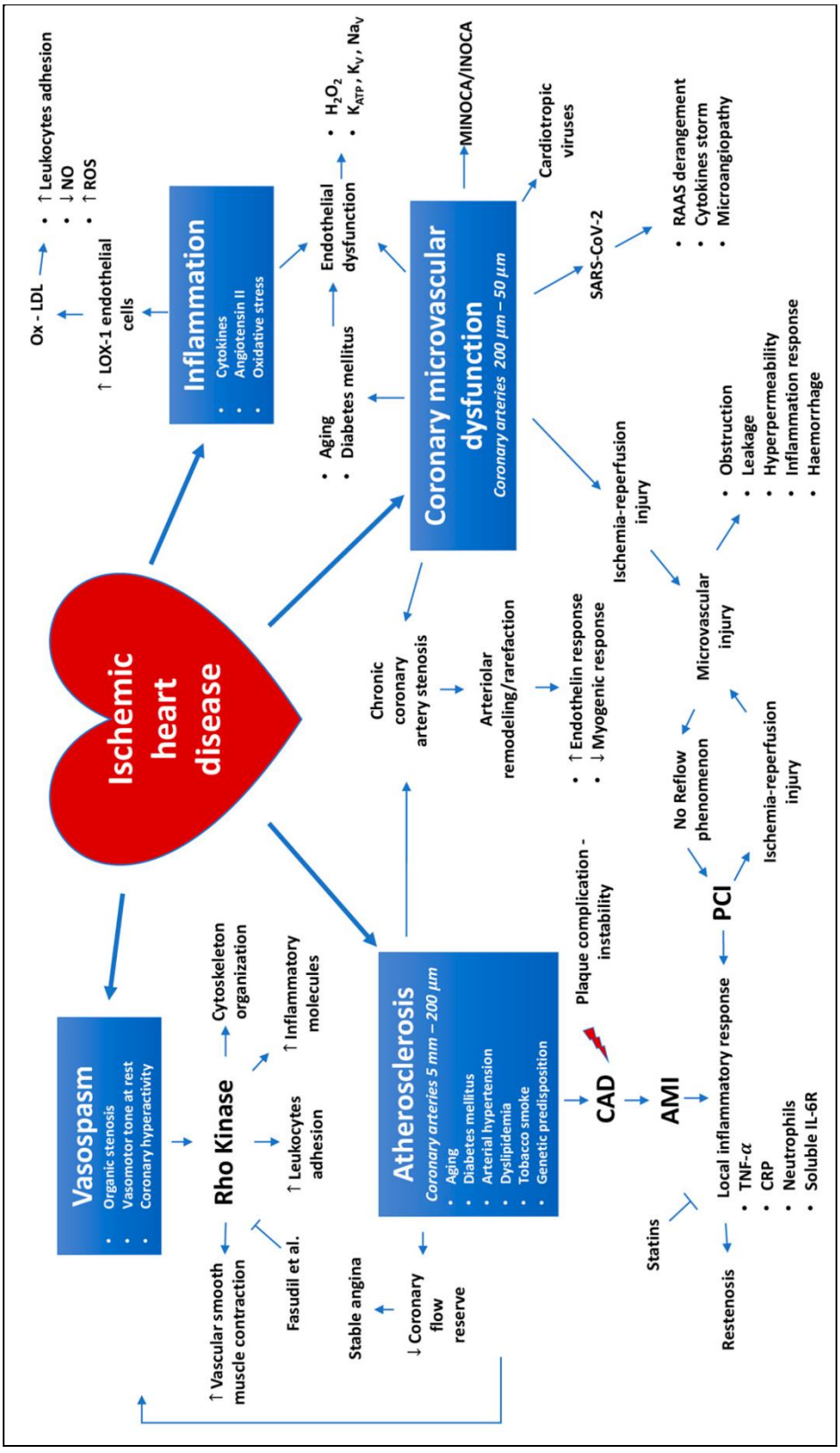
Ischemic heart disease is the condition of heart problems, caused by narrowed coronary arteries that supply oxygenated blood to the heart muscle. It is also known as coronary artery disease , occurs due to reduced blood flow to the heart muscle caused by the buildup of plaque in the coronary arteries, a condition known as atherosclerosis (Sharif *et al*., 2020). Ischemic heart disease is a pathophysiological condition caused by the disproportion between the myocardial oxygen demand and its supply.

Nutrition of the myocardium depends on the oxygen capacity of the blood and the amount of coronary flow (Vollmer-Conna *et al*., 2015). According to the European Society of Cardiology , CAD is defined as an episode of a reversible condition between the nutrient needs of the cardiac muscle and its demand that is associated with ischemia or hypoxia (Pająk, 2016). Coronary artery disease is the leading cause of death in both developed and developing countries (Khera *et al*., 2017). It is responsible for more than one-third of all mortality in people aged 35 and more (Benjamin *et al*., 2018). The most frequent cause of angina pectoris, acute coronary syndrome, and death globally is CAD (Ralston *et al*.,2022.). In the Asia-Pacific region, IHD is also the leading cause of death, accounting for almost half of the global burden of the disease (Nabovati *et al*., 2023).

Ischemic heart disease is a multifactorial disease and identifying its risk factors is of value in the prevention. Vital causes of IHD include: atherosclerosis, dissecting aneurysm, infective vasculists, syphilis, congenital defects, coronary artery spasm and migrating thrombus from deep venous thrombosis (DVT) (Judith, 2013).

Ischemic heart disease is a pathophysiological status brought on by the disparity amidst myocardial oxygen demand and its equipping, nourishment of the myocardium relies on the oxygen capacity of the bloodstream and the amount of coronary flow (Vollmer-Conna *et al*., 2015).

Ischemia can result from numerous mechanisms as shown in Figure (1), which include myocardial oxygen demand override its supply under some circumferences like escalate workload or decreased oxygen availability, coronary artery spasm which restricts blood flow, and intra-vascular clotting at the site of ruptured atherosclerotic plaque, which can limit coronary flow, sometimes, all these mechanisms happen concurrently, triggering the IHD (Kasprzyk *et al*., 2018).



Figure(1-1) Diagrammatic Illustration of the pathophysiological Processes behind Ischemic Heart Disease (Severino *et al*, 2020).

Ischemic heart disease relates to large coronary arteries, where stenosis (narrowing) reduces the reserves of coronary (in proportion to the degree of vasoconstriction), these stenosis may be coincide with a spasm expatiate its size, it is worth noting that acute coronary events resulting by formation of intravascular clot caused by ruptured atherosclerotic plaque (Frycz-Kurek *et al*., 2008).

Throughout acute ischemia, oxygen insufficiency blemishes cells ability to carry out oxidative phosphorylation of glucose and free fatty acids, so the prime exporter of energy turn into enzymic cytoplasmic glycolysis, epinephrine and norepinephrine secretion leading to escalate the hydrolysis of fats, that extends to the heart, due to the decrease in glucose supply, the main source of energy processing becomes the oxidation of free fatty acids, which negatively affects oxygen reserves, thus forcing the cells to anaerobically glycolysis, these events leading to accumulate of lactates and hydrogen ions, whether blood flow is not reinstate (reperfusion) to the heart muscle within a particular period, typically around 45 to 60 min, it can result in myocardium necrosis and subsequent cells death, usually referred to as a heart attack or myocardial infarction (Jankowski *et al*., 2018). Indeed, blood flow is adjusted by further mechanisms which include regulation of endothelium, myogenic regulation, neural and metabolism regulation to assure that heart draws sufficient perfusion.

The dilatation of the epicardia arteries is reliant on flow, and they experience shear stress that changes with each pulse. Across the coronary blood flow's phasicity, vasodilatation that is dependent on endothelial cells mediates the vasodilatory effects of shear stress (Severino *et al*., 2020).

Furthermore, hydraulic amendment of coronary arteries has been detected as people age (Nanayakkara *et al*., 2018). Aging is a physiological process and considered as an independent cardiovascular risk factor, and predispose IHD pathophysiology, as it could pursue with diabetes, arterial hypertension, dyslipidemia and tobacco smoke (Severino *et al*., 2020). Moreover, it is accountable for losing integrity of endothelial layer, arterial stiffness, loss elasticity and impair vascular adaptation to physical potency related to blood flow (Piccirillo *et al*., 2019).

What's more, aging promotes an increased expression of cyclooxygenase, thromboxane A, von Willebrand factor and factor VIII enhancing hypercoagulative state, also, inflammation response is participates in "hydraulic" modification of coronary arteries, assisting the arterial stiffness and growing of atherosclerotic plaque, where, macrophages are the key leukocytes engaged with the vascular damage that may also inducing by increasing age (Severino *et al*., 2020).

Modifiable risk factor which collect the most frequent factors that can be eliminated or minimized by changing the lifestyle such as stressful situation, smoking cigarette, elevated glucose, hypertension, visceral obesity, lipid disorders and thrombotic conditions (Essien *et al*., 2014).

A possible reason of IHD is that high blood pressure resulting from excess strain on coronary arteries serving the heart to slowly become narrowed from a buildup of fat, cholesterol and other substances, this build-up creates atherosclerosis, which later leads to accumulation of a blood clot or plaque and subsequently blood flow to the myocardium.(Gebremedhin & Gebrekirstos, 2021). Recent studies showed significant association between elevated TG/HDL-C ratio levels and ischemic heart disease, Authors reported significant correlation between

TG/HDL-C ratio and arthrosclerosis, and The TG/HDL-C ratio is associated with metabolic, inflammatory, and predictive of the severity of IHD (C. Chen & Dai, 2018), (Yunke et al., 2014), (Z. Chen et al., 2020).

Smoking cessation has the propensity to mitigate cardiovascular diseases and complications especially when achieved on a timely scale. Smoking cessation would reduce overall morbidity and mortality (Okorare *et al*., 2023). Overweight or obesity, physical inactivity; unhealthy diet and stressful condition are other risk factors of IHD (Hajar, 2017). Early detection and intervention regarding insulin sensitivity and other risk factors can play a pivotal role in preventing the onset or progression of IHD, thereby promoting public health and reducing the burden of cardiovascular disease (Park *et al*., 2021). In addition, age, along with sex is the most potent independent risk factor for atherosclerosis, though the sex gap vanishes after menopause, pre-menopausal women have lower illness rates than males do (Bosomworth and Khan, 2023).

In high-income nations, the number of fatalities linked to CVD has been reduced, but by 2030, it is expected that 23.6 million people would die from CVD-related causes worldwide, therefore, significant effort must be made to prevent and treat CVD, mostly through early targeting of cardiovascular risk factors by pharmaceutical or lifestyle therapies (Zhang *et al*., 2022).

2.2 Effect of lipids profile markers on ischemic heart disease

2.2.1 The effect of total cholesterol on ischemic heart disease

Cholesterol is a lipophilic molecule, which is crucial for human life and plays various essential roles in the body, it is a key component of the cell membrane, it participates to the structural makeup of the membrane as well as controls its fluidity, cholesterol considered as a precursor molecule in the synthesis of vitamin D, steroid hormones, and sex hormones, also it is integral component of bile salt (Di Ciaula *et al*., 2017).

Due to its functional importance, there are several sources for the body to obtain cholesterol, as it can be obtained from the digestion of dietary fats, and the body can also manufacture cholesterol from Acetyl-CoA and comes behind a serial of complex reactions (Huff *et al*., 2017).

Since cholesterol is mostly a lipophilic molecule, it does not dissolve well in the blood, for this reason, it is packaged in lipoproteins that have phospholipid and apolipoprotein (Wang *et al*., 2018).

While cholesterol is central to many healthy cell functions, it also can harm the body if it is reach to abnormal blood levels, interestingly, when LDL-cholesterol levels are too high, the condition referred to as hypercholesterolemia, the risk for premature atherosclerotic cardiovascular diseases increases (Ibrahim *et al*., 2023). Hypercholesterolemia is one of the major risk factors contributing to the formation of atherosclerotic plaques, these plaques lead to an increased possibility of various negative clinical outcomes, including, but not limited to, CAD, aortic aneurysms, and stroke (Huff *et al*., 2017). A study by Wald and Law (1995) showed that lowering total cholesterol (TC) would reduce the incidence of IHD by 60% for individuals aged 60 years, reduction of TC has been an integral part of public health campaigns, TC has also been a major part of

cardiovascular disease (CVD) risk prediction and prevention models (Hippisley-Cox *et al*., 2010).

For IHD prevention, "the lower, the better" cholesterol hypothesis has been accepted in the medical community for people with a high risk of heart disease (Trialists, 2010). Overall, control TC level becomes a relevant issue for East and Southeast Asia and Pacific island nations (Pirillo *et al*., 2021). The scrutiny of previous investigation proposes a strong and direct relationship between TC and IHD mortality (Lewington *et al.,* 2007; Kwon *et al.,* 2019). Exposure to elevated levels of LDL-cholesterol is one of metabolic risk factors accounted for 88% of deaths by IHD; about 44% of the global deaths by IHD can be attributed to high LDL- cholesterol (Pirillo and Norata, 2023). Evidence indicates that this relationship persists without a discernible threshold down to approximately 180–200mg/dL, but not below this range, in essence, these findings emphasize the importance of managing cholesterol levels within a certain range to mitigate the risk of IHD mortality , indeed, further investigations are required to provide detailed estimates of the relative risk associated with TC levels, which could help in the clinical and public health settings for IHD prevention and management (Kwon *et al.,* 2019).

2.2.2 The effect of triglycerides on ischemic heart disease

Triglyceride (TG) is a member of chylomicron compounds that sharing in buildup LDL and HDL-C (Jawameer and Saeed, 2021). Triglycerides serve as a concentrated energy source because they contain more than twice as much energy per gram as carbohydrates and proteins. This makes them an important fuel reserve for the body, particularly during times of fasting or increased energy demand, additionally, triglycerides are stored in adipose tissue and can be mobilized when energy is needed.

However, high levels of triglycerides in the bloodstream can be associated with an increased risk of cardiovascular disease (Thanassoulis *et al*., 2014).

Triglycerides are carried in chylomicrons and very-low-density lipoproteins (VLDLs), collectively termed TGRLs, although chylomicrons and VLDL particles are generally too large to cross the endothelium, triglycerides can influence several specific aspects of atherosclerotic lesion development (Farnier *et al*., 2021). triglyceride-rich lipoproteins (TGRLs) are subject to remodeling during intravascular lipolysis by the action of lipoprotein lipase (LPL), Inefficient clearance of TGRLs induces the formation of remnants, a decrease in LPL activity is associated with an excess of remnant particles, these remnants were considered as a causative agent for IHD (Varbo *et al*., 2014). In addition to their pro-inflammatory effects, TGRLs and their remnants promote endothelial dysfunction, and activate the coagulation cascade that leads to enhanced platelet aggregation, patients with elevated triglycerides have increased concentrations of thrombotic factors, such as fibrinogen and plasminogen activator inhibitor (Sandesara *et al*., 2019).

Finally, the rate at which TGRLs are produced is also influenced by metabolic factors–particularly insulin resistance–and many patients with elevated triglycerides have type 2 diabetes, metabolic syndrome or abdominal obesity, the secretion of TGRLs is enhanced with insulin resistance and increased concentrations of free fatty acids (Nordestgaard, 2016).

Hypertriglyceridemia, TG-rich lipoproteins, have revealed to be critical risk agent for CVD (Han *et al*., 2016). The role of serum TG as a screening test and a risk factor of IHD remain controversial (Aberra *et al*., 2020).

Fasting hypertriglyceridemia was associated with an increased risk of IHD at all levels of HDL-C, including high HDL-C levels thought to provide protection against IHD and fasting hypertriglyceridemia was a stronger risk factor than total cholesterol (Jeppesen *et al*., 1998). Several lines of evidence suggest that the association of plasma TGs with IHD is complex, however despite this consensus; uncertainty persists regarding the strength and independence of TG as a IHD risk factor (Jawameer and Saeed, 2021).

The study of Prospective Cardiovascular Munster (PROCAM) reported that the IHD risk increased proportionately with TG up to 800 mg/dl. The risk is associated with TG >200 mg/dl and was dependent on concomitant low HDL or elevated LDL to HDL ratio (Hopkins *et al*., 2005).

2.2.3 The effect of high density lipoprotein on ischemic heart disease

Lipoproteins are made up of a lipid core (which can contain cholesterol esters and triglycerides) and a hydrophilic outer membrane comprising phospholipid, apolipoprotein, and free cholesterol, they take excess cholesterol and return it to the liver for excretion (Huff *et al*., 2017).

High density lipoprotein (HDL) is a molecule that is antioxidant, anti-inflammatory, anti-apoptotic and increases macrophage cholesterol excretion and endothelial healing, the removal of cholesterol from the body by the liver via HDL is called reverse cholesterol transport; ATP-binding cassette transporter A1 (ABCA-1), ATP-binding cassette transporter G1 (ABCG-1), and Scavenger receptor class B1 (SR-B1) are effective in reverse cholesterol transport; ApoA1 and ApoA2 are mainly found in the structure of HDL, and also HDL includes apoCs, ApoE, apoD, apoJ, lecithin-cholesterol acyltransferase (LCAT), serum paraoxonase (PON1)

and platelet-activating factor acetylhydrolase (PAF-AH) molecules, enzymes carried by HDL prevent oxidative modification of LDL (Avci *et al*., 2018).

Pentraxin 3 (PTX-3) in HDL controls leukocyte level. Defective PTX-3 was associated with large atherosclerotic plaques and higher level of inflammation (Norata *et al*., 2010).

Clinically, HDL is significant since low HDL boost individuals' risk of atherosclerotic cardiovascular diseases (Karney *et al*., 2017; Sacks *et al*., 2017). While low HDL levels may indicate a higher risk of IHD, but increasing HDL levels has not been associated with a reduction in the incidence of IHD, consequently, further research is essential to elucidate the mechanisms involved and to determine the most effective strategies for reducing cardiovascular risk associated with HDL cholesterol levels, moreover, it underscores the importance of raising awareness about the role of HDL in preventing ischemic heart disease, potentially leading to improved preventive measures and interventions (Avci *et al*., 2018).

2.2.4 The effect of low density lipoprotein on ischemic heart disease

Low density lipoprotein is the particle that is responsible for transporting cholesterol to tissues. It is the primary carriers of cholesterol in blood because their main role is to deliver cholesterol to both peripheral and liver cells (Aherne *et al*., 2011). Cholesterol transportation is achieved by binding of the LDL receptor and apoB; There are three separate fractions of LDL: LDL (large/floating), IDL, and small dense LDL; The most atherogenic LDL is small dense LDL ,Notably LDL particles are thought to act as a major transporter of cholesterol; at least two-thirds of circulating cholesterol resides in LDL to the peripheral tissues(Huff *et al*., 2017) .

Clinically, LDL is significant since high LDL boost individuals' risk of atherosclerotic cardiovascular diseases (Karney *et al*., 2017; Sacks *et al*., 2017). It is a major contributor to develop atherosclerotic lesion (Huff *et al*., 2017).

Atherosclerotic plaques that play an essential role in acute coronary syndrome are divided according to their structural characteristics: plaque structure is with thin fibrous cap, dense necrotic core, high inflammatory cell density, and low smooth muscle content; it is called vulnerable plaque, vulnerable plaque increases with hypertension, diabetes mellitus, elevated LDL, decreased HDL (Avci *et al*., 2018). Past experiences indicate impact of hyperlipidemic therapy on the primary prevention of coronary artery disease (Packard *et al*., 1997; Downs *et al*., 1998).

2.3 Inflammatory processes and ischemic heart disease

Inflammatory processes play a major role in the development of vascular diseases, hence the importance of delving into the study of inflammatory markers prevalent in the cases of IHDA (Libby *et al*., 2011). Preceding epidemiological studies have mostly investigated the relations between some ‘downstream’ markers of inflammation such as C-reactive protein and fibrinogen with possibility to develop CAD (Danesh *et al*., 2005; Emerging Risk Factors Collaboration, 2010). However, human genetic studies had minimized the likelihood for these factors to be causally pertinent (Elliott *et al*., 2009; Wensley *et al*., 2011). In contrast, the inflammation ‘upstream’ markers such as cytokines, more likely to be directly etiologically pertinent to CAD, as they control the inflammation cascades (Kaptoge *et al*., 2014). Indeed, under physiological conditions, leukocytes are not stimulated by endothelia, even so, inflammation extremely shifts the nature of interaction between the endothelium and leukocytes, triggering the production of adhesion molecules that bind with

leukocytes, as a result leading to persistent and reinforcing a local inflammatory response, in turn local inflammation leads to produce several proteolytic enzymes that rupture the atherosclerotic cap, adaptive immune response has a critical role in the development of atherosclerotic lesion and their clinical aspects (Ammirati *et al*., 2015).

Randomized controlled trials were published in recent years have shed light on the intricate relationship between inflammation and residual risk in IHD, these insights have offered novel pattern for mitigating risk in individuals with IHD (Tong *et al*., 2020).

2.3.1 Pathophysiological role of cholesterol and its relationship with inflammation

Cholesterol, particularly low-density lipoprotein (LDL) cholesterol, plays a critical role in the development of atherosclerosis, which is characterized by the buildup of plaque in the arteries. This plaque buildup can lead to narrowing and hardening of the arteries, ultimately resulting in reduced blood flow to vital organs such as the heart, cholesterol deposition in the arterial walls triggers an inflammatory response, further exacerbating the progression of atherosclerosis, this inflammatory process involves the recruitment of immune cells, release of cytokines, and activation of inflammatory pathways, all of which contribute to plaque formation and instability (Das and Ingole, 2023).

Hypercholesterolemia and inflammation, concerning their synergistic biological contributions to atherosclerosis, and also their specific therapies, which now seem complementary approaches to reduce cardiovascular risk (Rocha and Santos, 2018). Dyslipidemia and inflammation are closely interconnected in their contribution to atherosclerosis, thus to IHD. In fact, LDL lowering drugs have anti-inflammatory effects (Tuñón *et al*., 2018). The Canakinumab Anti-

inflammatory Thrombosis Outcome Study (CANTOS) has shown that interleukin-1β (IL-1β) blockade reduces the incidence of cardiovascular events in patients with previous myocardial infarction and C-reactive protein levels >2 mg/L, these data confirm the connection between lipids and inflammation, as lipids activate the Nod-like receptor protein 3 inflammasome that leads to IL-1β activation. LDL-lowering drugs are the foundation of cardiovascular prevention (Ridker *et al*., 2017).

In IHD, the aspects of cholesterol metabolism and its inflammatory consequences have been widely described and it may counteract with several approaches, for example, statins and ezetimibe are important medication that were prescribed for reduction of LDL blood values and IHD related to hypercholesterolemia, additionally, statins have been shown to have anti-inflammatory effects, further contributing to their beneficial effects in atherosclerosis (Sabatine, 2019). Statins inhibit the enzyme hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase, which is involved in cholesterol biosynthesis. and inhibited by bempedoic acid, And the last, it is described to reduce LDL cholesterol when associated with statins (Ray *et al*., 2019). Proprotein convertase subtilisin/kexin type 9 (PCSK-9) is responsible for LDL receptor degradation and inhibits its migration to cells' membranes. For this reason, PCSK-9 inhibition reduces circulating LDL cholesterol and its related major cardiovascular events (Sabatine, 2019).

2.3.2 Inflammatory as a possible causal of ischemic heart disease

The myriad pathways by which inflammation can influence the atherogenesis and thus IHD are highly complex and dynamic, involving both local and systemic mechanisms (Libby *et al*., 2018). These mechanisms can be influenced by conditions such as autoimmune diseases, infections, and changes in host microbiota, but also by ambient pollution,

tobacco use, medications and other external factors (Libby *et al*., 2019). Moreover, there is evidence that this interplay is modulated by the genetic background (Fava and Montagnana, 2018). As aforementioned, different stimuli can lead to the activation of various cell types such as lymphocytes and mast cells, leading to expression of proinflammatory cytokines, which in turn further modulate the activity of monocytes which migrate from the bloodstream to the vessel wall, as well as of other cell types (Hansson, 2005).

As leukocytes migrate, the leukocytic infiltrate at the atheromatous plaque site can produce molecules such as proteases, pro-coagulant factors and inflammatory cytokines, further modulating thrombus formation and destabilization of the lesion, among the most important cytokines involved, a delicate balance between anti-inflammatory (such as interleukin-10 (IL-10) and pro-inflammatory (such as IL-18 and IL-1 and, downstream, IL-6) signaling has a crucial role (Libby *et al*., 2018; Libby *et al*., 2019; Ridker *et al*., 2020).

In this balance, NLR family pyrin domain containing 3 (NLRP3) inflammasome is a crucial component of the innate immune system and has garnered significant attention in recent years, it is a large protein complex involved in detecting various stimuli, including pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs), these stimuli can arise from diverse sources such as cholesterol accumulation, hypoxia, or dysregulation in autophagy processes (Mach *et al*., 2020).

Another issue to be considered is the production of autoantibodies including cardiac autoantibodies (Libby *et al*., 2018). These may be related to background autoimmunity (as in the case of systemic lupus

erythematosus) and further amplify the immune response (Frostegård, 2005). But can also be found in individuals with CVD as well as in the general population, thus further illustrating the overlap between mechanisms (Vilela *et al*., 2017). In addition, data support the notion that there are changes in the leukocyte profile in CVD (Groot *et al*., 2020). Although the full scope of these findings remains to be fully ascertained, a pro-inflammatory imbalance as expressed by changes in the neutrophil-to-lymphocyte ratio, suggesting a shift towards increased inflammatory mediators via neutrophils and a reduction in anti-inflammatory signaling via lymphocytes, has been proposed as among the mechanisms underlying the association between changes in blood cell profiles and CVD (Angkananard *et al*., 2018).

Emerging non-traditional risk factors (e.g., oxLDL and LOX-1) seem to playing important roles in IHD (Steinberg, 2002; Suzuki *et al*., 2002). The oxLDL is implicated in various stages of atherogenesis, from plaque formation to destabilization, It contributes to endothelial dysfunction, stimulates reactive oxygen species (ROS) generation, inhibits nitric oxide (NO) synthesis, enhances monocyte adhesion to endothelial cells, induces vascular smooth muscle cell (VSMC) migration and proliferation, and leads to foam cell formation (Mehta and Li, 2002). Moreover, oxLDL can induce apoptosis and necrosis of vascular endothelial cells, VSMCs, and macrophages (Wu *et al*., 2014). Elevated oxLDL levels correlate with plaque instability and severity of myocardial ischemia in coronary atherosclerotic lesions (Ehara *et al*., 2001). These proceedings are regulated by the overexpression of the lectin-like oxidized LDL (LOX-1), a scavenger receptor, plays a crucial role in the uptake of oxLDL into endothelial cells, its expression is modulated by various factors including cytokines, mechanical forces, angiotensin II, oxidative

stress, and directly by oxLDL (Wang *et al*., 2015). Overexpression of LOX-1, possibly induced by oxLDL, contributes to endothelial cell apoptotic death, mediated by the overproduction of ROS, This overproduction of ROS leads to the generation of highly reactive oxidants like peroxy-nitrite (ONOO−), which are toxic to endothelial cells and promote apoptosis (Mollace *et al*., 2015).

2.3.3 Inflammation as consequence of ischemic heart disease

Acute myocardial infarction (AMI) produces a significant local inflammatory response, which starts in the myocardium and propagates systemically through the blood stream, lots of inflammatory cytokines, such as tumor necrosis factor alpha (TNFα) and various chemokines that are weakly represented in healthy hearts, reach high levels during myocardial infarction (Aker *et al*., 2003). Increased peripheral white blood cell counts, particularly neutrophil counts, and elevated acute phase reactant levels, such as CRP, are typical during ACS (Barron *et al*., 2000; Bursi *et al*., 2007). Increased peripheral blood neutrophil counts have been linked in many studies to short-term unfavorable outcomes following ST-elevation myocardial infarction (STEMI), such as death(O'Donoghue *et al*., 2008).

Within the biological atherothrombosis linked to the IL-6 pro-inflammatory pathway, interleukin-1β (IL-1β) induces VSMC hypertrophy and proliferation, it promotes leukocyte recruitment and acts as a procoagulant to the walls of the vessels, Canakinumab, a human monoclonal antibody that targets IL-1β, decreases inflammatory response, atherothrombosis, and cardiovascular events because of this. Without changing cholesterol levels, it lowers plasma CRP and IL-6 in patients with a history of myocardial infarction (Ridker *et al*., 2017).

Furthermore, significant cardiovascular events and post-ischemic chronic heart failure are linked to lower blood vasostatin-2 levels (Pan *et al*., 2016). Plasma levels of the galectin-3 binding protein are linked to long-term death in CAD, regardless of the shape of the plaque (Gleissner *et al*., 2016). Here, statin medication appears to lessen leukocyte and neutrophil cell numbers as a result of the myocardial infarction, hence lowering the cellular inflammatory response (Pourafkari *et al*., 2016). Percutaneous coronary intervention (PCI) may also, in addition to other variables including the potential interaction between stent materials and passive red blood cells, because a local inflammatory response that contributes to restenosis electrical characteristics of cell membranes (Basoli *et al*., 2012).

Multiple molecular signals are the source of inflammation. Innate immune responses including neutrophils, ROS, toll-like receptors, myeloperoxidase, and interleukins are initiated by injured cardiomyocytes (Čermák *et al*., 2016; Huang *et al*., 2016). The myocardial ischemia-reperfusion damage is a critical situation, ironically, the process of cardiac reperfusion can actually cause cardiomyocyte death, the myocardium may become exposed as a result of post-ischemia intracellular edema, which is the inflammatory reaction to the acute ischemic shock to harmful consequences of ROS during ischemia-reperfusion (Francone *et al*., 2009).

Furthermore, during reperfusion, free fatty acids significantly rise and their harmful effects on cellular membranes cause arrhythmias and a decline in heart function (Lavalle *et al.,* 2020). Low-grade endotoxemia and thrombus development in relation to an unstable coronary plaque may be related; in addition patients with STEMI had higher amounts of lipopolysaccharide (LPS), an endotoxin formed from *Escherichia coli,* and other inflammatory products such as tissue factor, CPR, and numerous

cytokines than patients in the control group or those with stable angina, Obtained from *Escherichia coli Zonulin* and *P-selectin* levels positively correlate with LPS levels, indicating a potential function for the gut microbiota in coronary thrombosis following translocation into the systemic circulation, however, because the increased levels of LPS in STEMI patients may be a result of inflammation associated to the infarction rather than the cause, the relationship between *Escherichia coli*-derived LPS and STEMI is not entirely clear-cut from a myocardial attack. Rather than being the main cause of IHD, LPS and inflammation may destabilize atherosclerotic plaques and contribute to their progression together with other variables, given the complexity of IHD pathophysiology (Carnevale *et al*., 2020).

2.4 Effect of immunological factors on ischemic heart disease

2.4.1 Effect of interleukin-6 on ischemic heart disease

Interleukin-6 (IL-6) was firstly discovered and cloned in the 1980s by the Kishimoto's laboratory Hirano *et al*., 1989 as a small glycoprotein that can be produced by a variety of cells, and responds to various stimuli (Hirano, 1998). IL-6 is a pro inflammatory cytokine, which is mainly expressed by leukocytes, especially by activated macrophages but also by endothelial cells and smooth muscle cells (Thomsen *et al*., 2016). IL-6 is the founding member of the IL-6 cytokine family, which also includes IL-11, IL-27, IL-30, IL-31, leukemia inhibitory factor (LIF), oncostatin M (OSM), cardiotrophin-like cytokine (CLC), ciliary neurotrophic factor (CNTF), cardiotrophin-1 (CT-1), and neuropoietin , IL-6-IL-6 R alpha complex promotes gp130 dimerization and the formation of a heterohexameric complex (Feng *et al*., 2022).

A large number of studies have confirmed that IL-6 has both pro-inflammatory and anti-inflammatory effects via different IL-6Rs, the receptor complexes of IL-6 are composed of IL-6R or soluble IL-6R and gp130, it seems that the pro-inflammatory effect mainly relies on trans-signaling mediated by sIL-6R and that the anti-inflammatory effect mainly depends on membrane-bound IL-6R (Heinrich *et al*., 2003; Jones and Jenkins, 2018).

The signaling pathways of IL-6 family members are similar but distinct because of their similar but distinct receptor complexes. One major signaling pathway is the activation of Janus kinase (JAK) tyrosine kinase family members, leading to the activation of the signal transducers and activators of transcription (STAT) transcription factors, mostly STAT3, Another major signaling pathway is the JAK-SH2 domain tyrosine phosphatase 2 (SHP2)- mitogen-activated protein kinase (MAPK) pathway (Yan *et al*., 2016).

Interleukin-6 (IL-6) is an acute-phase protein that plays a significant role in the inflammatory response, vascular inflammation, and atherosclerosis process (Heinrich *et al*., 1990). Besides, IL-6 involved in chronic inflammation; It's one of the few cytokines significantly play a role in both acute and chronic inflammation (Feghali and Wright, 1997; Baker *et al*., 2019).

The biological functions mediated by IL-6 are very complicated, which after the organism is stimulated by tissue injury, infection and inflammation, IL-6 could induce the production of cytokines including acute phase reaction proteins via NF-Kb, by activating endothelial cells and increasing the expression of adhesion molecules and the secretion of chemokines, IL-6 induces neutrophils to regroup into the affected tissue

(Romano *et al*., 1997). After antigen stimulating B cells, IL-6 could induce B cells to proliferate, differentiate and produce antibodies (Yao *et al*., 2014). IL-6 could also induce the proliferation and differentiation of thymic T cells, activate macrophages (Chen *et al*., 2017) and natural killer cells, and participate in the coordination of immune system to resist harmful stimuli. IL-6 is related to tissue fibrosis and vascular endothelial injury, promotes angiogenesis and increases vascular permeability (Tanaka *et al*., 2018) by stimulating the proliferation and migration of circulating endothelial progenitor cells (Fan *et al*., 2008), and also participates in the proliferation and migration of SMCs ,Once IL-6 levels are abnormally elevated, the physiological disorder would occur, which leads to a series of pathological changes including inflammatory injury, plaque formation and rupture, and thrombosis. These changes have a promotional effect on the development of CAD (Ikeda *et al*., 1991).

IL-6 is indeed a key player in the development and progression of atherosclerosis, a condition characterized by the buildup of plaque in the arteries. Atherosclerosis is a major underlying cause of ischemic heart disease, including conditions such as CAD and heart attack (Okazaki *et al*., 2014). IL-6 plays an important role in regulating the downstream inflammatory responses that contribute to the development of atherosclerosis, IL-6 perpetuates vascular inflammation by promoting smooth muscle cell (SMC) proliferation and migration, endothelial dysfunction and the recruitment and activation of inflammatory mediators, which result in atherosclerotic plaque development and plaque destabilization (Hartman and Frishman, 2014). Clinical data have shown that elevated levels of IL-6 are associated with an increased risk of mortality from unstable coronary artery disease, This underscores the significance of IL-6 as a biomarker for assessing the severity and prognosis

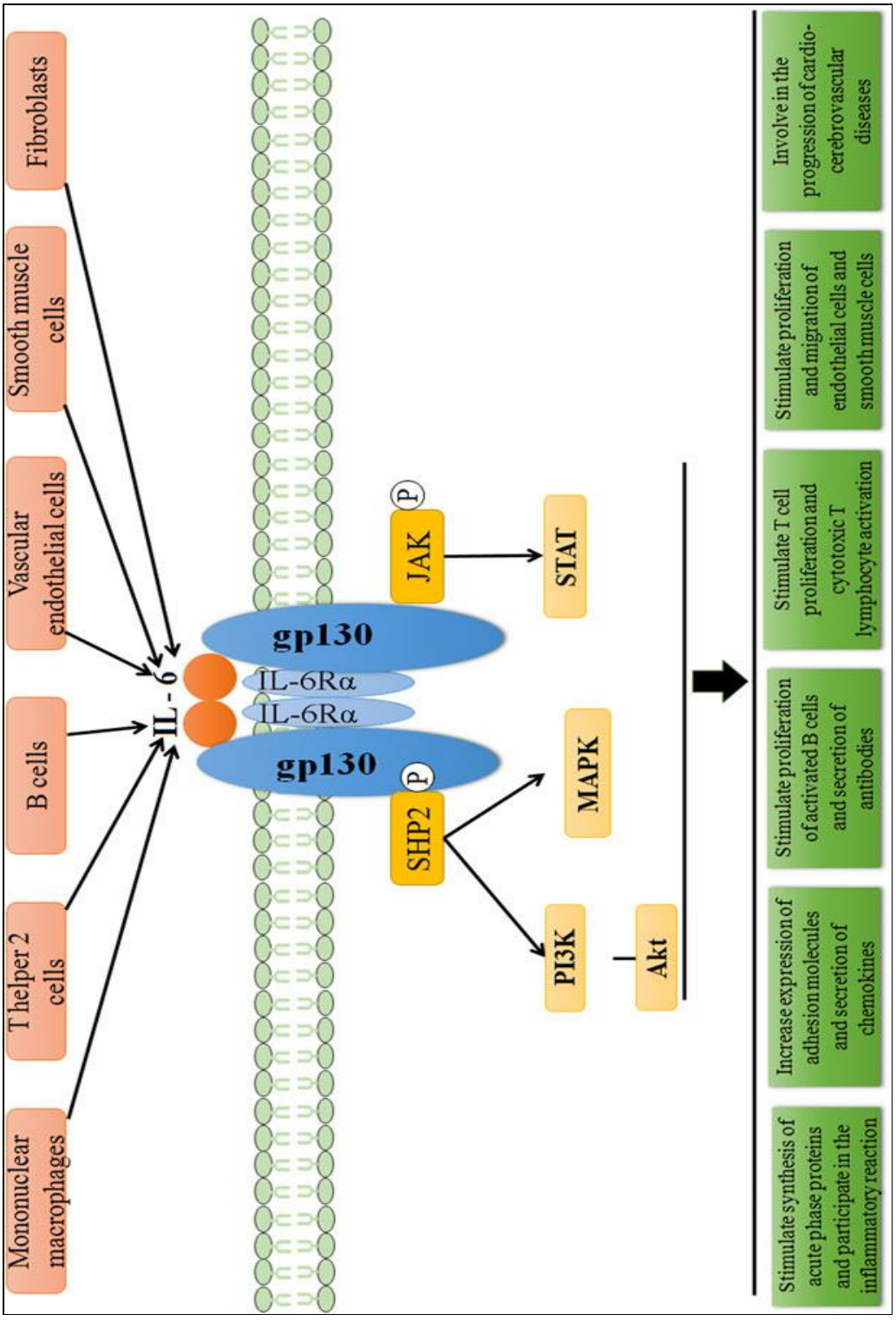
of cardiovascular disease , overall, continued research into the role of IL-6 in the pathogenesis of ischemic heart disease holds promise for advancing our understanding of disease mechanisms and developing novel therapeutic strategies to improve patient outcomes (Feng *et al*., 2022).

2.4.2 Effect of soluble interleukin-6 receptor on ischemic heart disease

The combination of IL-6 and IL-6R can activate downstream signal transduction pathways (Figure 2), soluble IL-6R (sIL-6R) was recognized in several body fluids, it binding with IL-6 in pathway called trans-signaling (Muhi et al., 2023).

In human, two vital mechanisms by which, sIL-6R is produced, first one involves cleavage of membrane-bound IL-6R by proteases such as ADAM10 and ADAM17, cleavage by ADAM10 is basic, while that of ADAM17 is trigger by pro inflammatory cytokines (Mülberg *et al*., 1993). The second one relies on alternative splicing of IL-6R mRNA resulting in generation of IL-6R lacking the transmembrane and cytosolic domains (Lust *et al*., 1992).

In circulation, IL-6 can interact with sIL-6R and the IL-6/sIL-6R complex, (also designated Hyper-IL-6), links a membrane bound gp130, triggering a pathway identical to the pathway in the classical signaling that was discussed earlier (Villar-Fincheira *et al*., 2021).



Figure(1- 2) Schematic Representation of IL-6 Signal Transduction (Su *et al.*, 2021)

Activation signaling of IL-6 by soluble form of the IL-6R differs from IL-6 signaling by pre-existing membrane-bound receptors (classic signaling only occurs in immune cells and in hepatocytes), for activation of other cells, IL-6 needs to form a complex with the sIL-6R, this complex will then activate cells through binding to gp130 expressed on the surface of cells (trans-signaling) (Baran *et al*., 2018). This is different from most other cytokines for which soluble receptors generally act as decoys inhibiting activation of cells, for IL-6 this function is instead served by soluble gp130 (sgp130) that can inhibit the binding of circulating IL-6/IL-6R complexes to membrane-bound gp130 (Scheller *et al*., 2011),The trans-signaling pathway is active in almost any cell type.

The soluble gp130 (sgp130) is available and it is manufactured either by shedding using ADAM10 and ADAM17 preferentially or by alternative splicing, Sgp130 binds with the complex of IL-6/sIL-6R but does not bind with IL-6 alone, so, the sgp130 job is to catch the complex of IL-6/sIL-6R selectively, trans-signaling mediates IL-6 pro inflammatory actions (Villar-Fincheira *et al*., 2021). When IL-6 engaged with their unique sIL-6R, it is become able to activate Janus kinase (JAK)-STAT pathway, SHP2-mitogen-activated protein kinase (MAPK) pathway and phosphoinositide 3-kinase (PI3K)-protein kinase B (Akt) pathway (Akbari and Hassan-Zadeh, 2018). Among which, JAK1-STAT3 pathway is the main signaling pathway in the IL-6 family of cytokines (Taniguchi and Karin, 2014). IL-6 first binds to IL-6Rα to form a dimer, which is activated by phosphorylation, and then forms an activated trimer complex with gp130 (Rose-John, 2017). Which initiates the intracellular signal cascade and induces the phosphorylation of JAK family related to IL-6R, further activating the downstream transcription factors of STAT family and binding to the promoter region of target genes, to generate a series of

essential functions to maintain normal vital movements, such as inflammation, immune responses and cell recruitment, various IL-6 target genes are caused by activation of the transcription factor STAT3, which also stimulates the expression of genes encoding suppressor of cytokine signaling-1 (SOCS1) and SOCS3, in addition, SOCS1 can bind tyrosine phosphorylated JAK, whereas SOCS3 binds tyrosine-phosphorylated gp130 to terminate IL-6 signaling through a negative feedback loop (Naka *et al*., 1997).

Similarly to C-reactive protein and fibrinogen, whose synthesis is stimulated by IL-6R signaling, high circulating concentrations of IL-6 were associated with increased risk of CAD events in prospective observational studies (Ridker *et al*., 2000; Sattar *et al*., 2009). On the basis of mendelian randomization studies IL6R signaling could be an important therapeutic target for prevention of CAD (Keavney *et al*., 2006).

The IL6R variant and elevated IL-6 levels associated with reduction in coronary risk, in a genetic study, individuals with the IL6R variant exhibit reduced activity in the signaling pathway associated with IL-6, this leads to less severe consequences typically associated with IL-6 activity, which may ultimately result in a lower risk of coronary issues, this explanation aligns with the observed effects of pharmacological intervention with tocilizumab, which also inhibits IL6R signaling and has been associated with similar outcomes in clinical settings (Interleukin-6 Receptor Mendelian Randomisation Analysis (IL6R MR) (Consortium, 2012). Despite IL-6 is well known as a marker of IHD risk both in the general population and in subjects with prevalent CAD, much less is known about the relationship of sIL-6R with CAD risk and if the association between elevated IL-6 and increased CAD risk is dependent on classical or trans-activation of IL-6 signaling, a better understanding of the

respective roles of IL-6 and sIL-6R in CAD is important because available therapies target different types of IL-6 signaling , accordingly, IL-6 signaling is receiving increasing attention as a possible target for intervention in subjects with cardiovascular disease and signs of residual inflammation (Ridker and Rane, 2021).

2.4.3 Effect of Tumor Necrosis Factor-α on ischemic heart disease

TNF-α is originally discovered during 1975 that could kill mouse tumor cells, which is why we call it "tumor necrosis factor") (Carswell *et al*., 1978). It belongs to the TNF superfamily of proteins consisting of 157 amino acids and is mainly generated by activated macrophages, T-lymphocytes, and natural killer cells (Horiuchi *et al*., 2010), but several subsequent studies have shown that it is also produced by nonimmune cells such as endothelial cells, adipocytes, neurons, and myocardial cell (Riezzo *et al*., 2009). TNF-α exists in two forms: transmembrane (tmTNF-α) or soluble TNF- α (sTNF-α). tmTNF-α is expressed on the surface of activated lymphocytes, macrophages, and other cell types, and when processed by TNF-α-converting enzyme, it is released as the sTNF-α (Jiang *et al*., 2017).

The biological activity of TNF-α is achieved by two receptors: TNF-α receptor1 (TNFR1) and TNF-α receptor 2 (TNFR2) (Tartaglia *et al*., 1991). TNFR1 is expressed in most nucleated cells, and it is fully activated by both tmTNF-α and sTNF-α (Tracey *et al*., 2008). TNFR2 is expressed mainly in immune cells but also in myocardial cell and is primarily activated by tmTNF-α in the context of cellto-cell interactions (Keck *et al*., 2019). TNF-α is involved in many pathophysiological processes, such as inflammation, immunity, cell proliferation, apoptosis, and lipid metabolism (Waters *et al*., 2013). Abnormal secretion of TNF-α leads to various

diseases, such as rheumatoid arthritis (RA), inflammatory bowel disease, spondylarthritis, psoriasis, noninfectious uveitis, and CAD (Qian *et al*., 2022). TNF-α production is induced in response to various stimuli, including ischemia, oxidative stress, and inflammation, in the context of ischemic heart disease, TNF-α has been implicated in several pathological processes from endothelial cell dysfunction to myocardial infarction, TNF-α is widely involved in the occurrence and development of CAD (Clark *et al*., 2015; Lu *et al*., 2020; Qian *et al*., 2022).

As a chronic inflammatory disease, TNF-α-involved vascular inflammation plays an important role in the progression of CAD (Jia et al., 2022). In local inflammation, TNF-α is released by inflammatory cells, endothelial cells, and cardiomyocytes (Riezzo *et al*., 2009; Horiuchi *et al*., 2010). It then mediates endothelial dysfunction, foam cell formation, angiogenesis, smooth muscle proliferation, and thrombosis. In vascular homeostasis, vascular endothelial cells act as a barrier, so any disruption for this barrier leads to inflammatory cell invasion, which contributes to a variety of vascular diseases, including atherosclerosis (Clark *et al*., 2015; Garcia *et al*., 2018). In the development of atherosclerosis, endothelial cell apoptosis plays an important role in the regulation (Duan *et al*., 2021). TNF-α induces endothelial cell apoptosis by upregulating autophagy, which is inhibited by arachidonic acid (Chen *et al*., 2021). In addition to apoptosis, endothelial cell senescence is positively associated with the development of atherosclerosis, thus progression of IHD (Honda *et al*., 2021).

In the early stages of atherosclerosis, monocytes migrate to the intima of coronary artery and differentiate into macrophages (Zhang *et al*., 2017). When oxidized low density lipoprotein (ox-LDL) intake exceeds the metabolic capacity of macrophages, macrophages transform into foam

cells (Cao *et al*., 2019). Foam cells are involved in fatty streak formation, a hallmark of the early stages of atherosclerosis (Zhang *et al*., 2020). The was study conducted by (Oberoi *et al*., 2016) confirms that TNF-α promotes monocyte adhesion to endothelial cells, which is effectively blocked by adalimumab. ox-LDL induces oxidative stress and increases TNF-α secretion by macrophages via reducing the inhibition effect of miR-491- 5p on matrix metalloproteinase 9 (Cao *et al*., 2019). Meanwhile, TNF-α enhances the uptake of ox-LDL by macrophages in a concentration-dependent manner given its multifaceted effects on inflammation, endothelial function, smooth muscle cell behavior, apoptosis, and matrix remodeling, TNF-α is considered to play a significant pathological role in ischemic heart disease, targeting TNF-α or its downstream signaling pathways may represent a potential therapeutic strategy for the management of IHD, although the clinical efficacy and safety of such interventions require further investigation (Ding *et al*., 2018).

Chapter Two

Materials and Methods

2. Supplies and Methodology

2.1.1 Tools and Apparatuses

The instruments that used in the present study are listed in table(2.1) with their supplier.

Table (2.1) Instruments and their supplier

NO.	Equipment and Tools	Suppliers
1.	Automatic Micropipette (100-1000µL)	Humapette -Germany
2.	Centrifuge	Kokusan-H-19f ---Japan
3.	Deep freezing	GFL-Germany
4.	Distillation Apparatus	Lab tech – Korea
5.	ECG iE-300	Biocare-China
6.	EDTA Tubes	AFCO-DISPO –Jordan
7.	ELISA (Reader and washer)	PARA MEDICAL-Italy
8.	Eppendorf Tubes (1.5ml)	Firatmed - Turkey
9.	GE-portable Healthcare ultrasound system	General Electric –U.S.A
10.	Gel Tubes	AFCO-DISPO –Jordan
11.	Incubator (model IB -909)	Memmert,Co.Ltd,Germany
12.	Latex Gloves	ARISS-Thailand
13.	Medical Syringe	Shengguang – China
14.	Mendray-BS-240Pro	Shenzhen Mindray Bio-Medical Electronics-Germany
15.	MDF-Sphygmomanometer	MDF-U.S.A
16.	Physician Balance Beam scale	Health O meter-U.S.A
17.	Refrigerator	Hitachi-Malaysia

2.1.2 Biological and chemical Requirements:

Table (2.2) contain a list of chemicals and biological materials, and manufactures that were employed in current investigation.

Table (2.2) Biological and chemical Requirements in present study

No.	Chemicals	Supplier
1.	Ethanol 70%	Scharlau – Spain
2.	Kit for Interleukin 6(Human) ELISA-IL6	Bioassay technology Laboratory- China
3.	Humans Kit for soluble Interleukin 6 receptor, sIL-6R ELISA-Kit	Bioassay technology Laboratory- China
4.	Human tumor necrosis factor α , TNF-α ELISA -Kit	Bioassay technology Laboratory- China
5.	HDL-cholesterol Kit	Shenzhen Mindray Medical Bio-Electronics-Germany
6.	LDL-cholesterol Kit	Shenzhen Mindray Medical Bio-Electronics-Germany
7.	Total cholesterol Kit	Shenzhen Mindray Medical Bio-Electronics-Germany
8.	Triglycerides kit	Shenzhen Mindray Medical Bio-Electronics-Germany

2-2 Methodology:

2-2-1 The study Sample

This study included 90 individuals of both sexes, including 60 patients of IHD (30 males,30 females) group and 30 individuals randomly selected as controls group. They were visiting (Imam –Al-Hussien-Medical city ,Imam AL-Hassan AL-Mujteba hospital) in Karbala province .The ages of

both groups ranged from (40-85 years) , and samples were collected from 1/ December /2022 until 1/May/2023.

2-2-1 Ethics Statement

All patients who enrolled in this study agreed on ethical considerations with initial approval for this study at the ischemic heart disease at Imam – Al-Hussien-Medical city ,Imam AL-Hassan AL-Mujteba hospital in in Karbala province .Before starting the study ,approval from the Ministry of Health was obtained ,and the studýs objective was explained to each participant .

2-2-3 Inclusion Criteria

All patients who suffer from ischemic heart disease have hypertension without diabetes ,and range Age (40-85 years)

2-2-4 Exclusion Criteria

All patients who suffer ischemic heart disease with hypertension and diabetes.

2-2-5 Blood Collection

All specimen were collected in Karbala (Imam-AL-Hussian-medical city, Imam Al-Hassan Al-Mujteba hospital), at period from December-2022 to May-2023. A total of 90 individuals of both sexes, comprising 60 individuals(30 males,30 females) as patients group with age range 40-85 years, furthermore, 30 individuals (15 males ,15 females) randomly selected as control group with age range 40-85 years. A questionnaire form (Appendix-1) was used to record special note. Each individuals vein was punctured to obtain five ml sample of blood in sterile EDTA tube. To separate serum, let blood sample for clotting at room temperature before centrifugations process at 3000 round / minute for 10-15 minutes , then separated into multiple 300 µl aliquots and stored in Eppendorf tubes at -

20 ^{o}C in Deep freeezing until used and other serum used to lipid profile analyses. Several investigation were done on serum included: IL-6, sIL-6R, TNF-α as listed in figure (2.1).

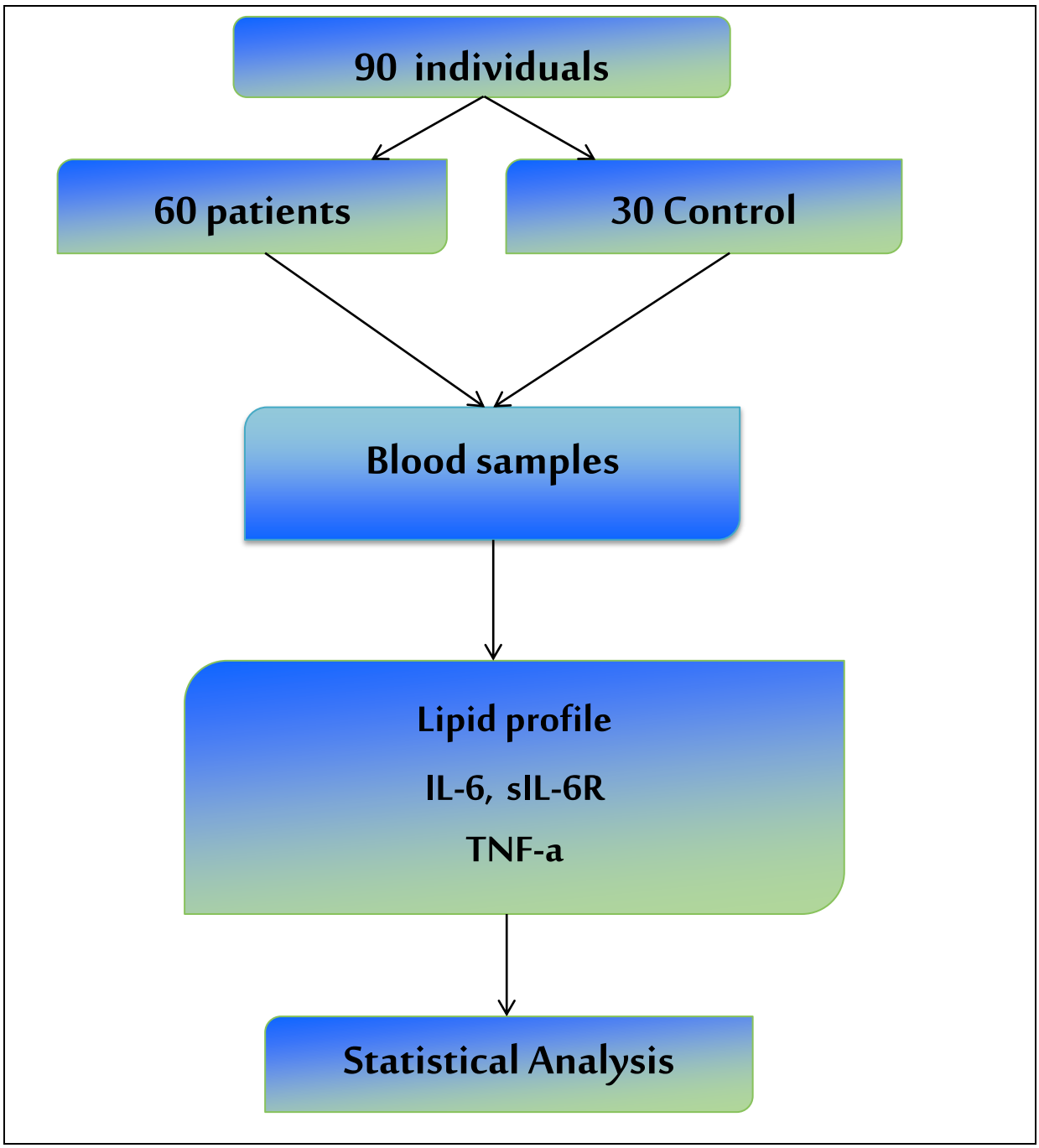


Figure (2.1): Study Design

2.2.6 Calculation of body mass Index (BMI)

Body mass index it is a numerical value of the body weight divided by the length square and extracted by mathematical calculation (Jan and Weir.,2019)

BMI =Weight (Kg) / Height (M^2)

They were classified into three groups:

1-The BMI of normal weight range is (18.5-24.9) Kg/M^2

2- The BMI of overweight range is (25-29.9) Kg/M^2

3- The BMI of obese is ≥ 30 Kg/M^2

2.2.7 Estimating the Levels of Biochemical Parameters

2.2.7.1 Estimation of Triglyceride (TG)

The level of TGs was estimated according to the manual procedure of kit Shenzhen Mindray Bio- Medical Electronics- Germany (normal triglyceride ≤ 200.0 mg/dL (Rifai,*et al.*, 1999).

2.2.7.1.1 Reaction Principle

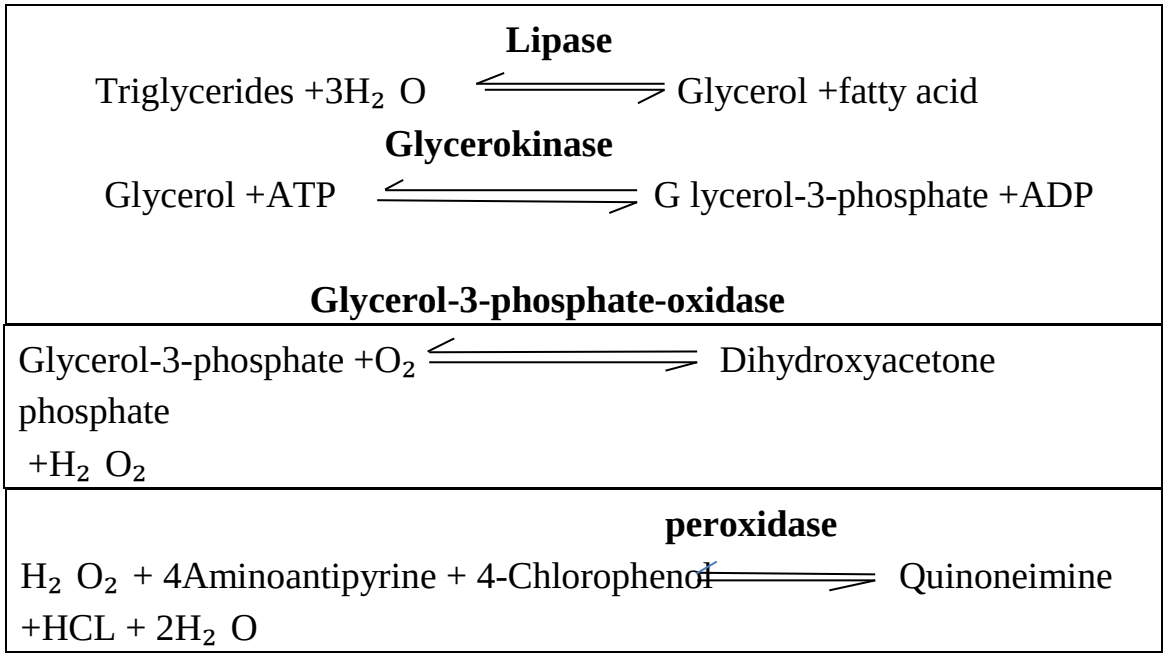


Figure (2-2) Measurement of triglyceride concentration (Rifai N,*et al.*, 1997).

Triglycerides are catalyzed by Glycerol-3-phosphate-oxidase(GPO), lipase and Glycerokinase (GK) through series steps of enzymatic catalysis that result in H_2 O_2 ,which oxidize-4-Aminoantipyrinel to generate a colored dye of quinonimine. There is correlation related in absorbency increase with concentration of triglycerides .

Reagents

Table (2-3) Reagents of Triglycerides Kit (Shenzhen Mindray Bio-Medical Electronics-Germany)

Components and concentrations		
	phosphate buffer	50mmol/L
R:	4-Chlorophenol	5mmol/L
	ATP	2mmol/L
	Mg_2	4-5mmol/L
	Glycerokinase	≥ 0.4U/mL
	Peroxidase	≥ 0.5U/mL
	Lipoprotein lipase	≥ 1.3U/mL
	4-Aminoantipyrine	0.25 mmol/L
	Glycerol-3-phosphate-oxidase	≥ 1.5U/mL

2.2.7.1.2 Assay Procedure

Table (2-4) Assay procedure of Triglycerides Kit (Shenzhen Mindray Bio- Medical Electronics-Germany)

	Blank	**Sample**
R	1000µL	1000µL
Dist.water	10µL	-
Sample	-	10µL
Mixed thoroughly at 37℃, and read the absorbance 10 min. later.		
ΔA=[ΔA sample] - [ΔA blank]		
Δ Absorption(AU) **=Δ Absorption sample –Δ Absorption blank**		
At 510 nm.		

2.2.7.2 Total Cholesterol (TC)

The level of TC was estimated according to the manual procedure of kit Shenzhen Mindray Bio- Medical Electronics-Germany .normal total cholesterol ≤ 200.0 mg/dL (Rifai,*et al.*, 1999).

2.2.7.2.1 Reaction Principle

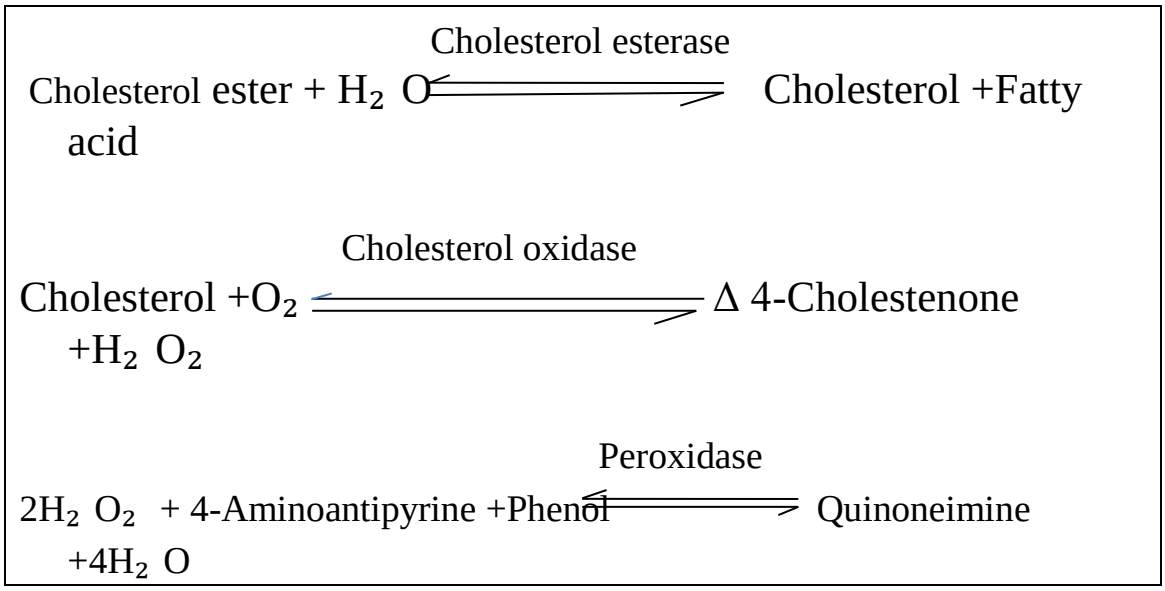


Figure (2-3) Measurement of Total cholesterol concentration (Rifai N,*et al.*, 1997).

cholesterol ester is catalyzed by Cholesterol esterase (CHE) and cholesterol oxidase (CHO) to produce H_2O_2 , which oxidates 4 Aminoantipyrine with phenol to produced colorful stain quinoneimine. An increase of absorbency is correlated directly to the amount of cholesterol. At the absorbance 510 nm .

Reagents

Table (2-5) Reagents of total cholesterol Kit (Shenzhen Mindray Bio-Medical Electronics-Germany)

Components and concentration	
Phosphate buffer	100mmol/L
phenol	5mmol/L
4-Aminoantipyrine	0.3mmol/L

Cholesterol esterase	> 150kU/L
Cholesterol oxidase	> 100kU/L
Peroxidase	5kU/L

2.2.7.2.2 Assay Procedure

Table (2-6) Assay procedure of total cholesterol Kit (Shenzhen Mindray Bio- Medical Electronics-Germany)

	Blank	**Sample**
R	1000µL	1000µL
Dist.Water	10µL	-
Sample	-	10µL
ΔA=[ΔA sample] - [ΔA blank]		

2.2.7.3 Evaluation of High-Density Lipoprotein cholesterol(HDL-C)

The estimation HDL-C level ,was depending on procedure manually Shenzhen Mindray Bio- Medical Electronics-Germany normal High-Density Lipoprotein cholesterol ≥ 35.0 mg/dL (Rifai,*et al.*, 1999).

2.2.2.3.1 Principle of the Assay

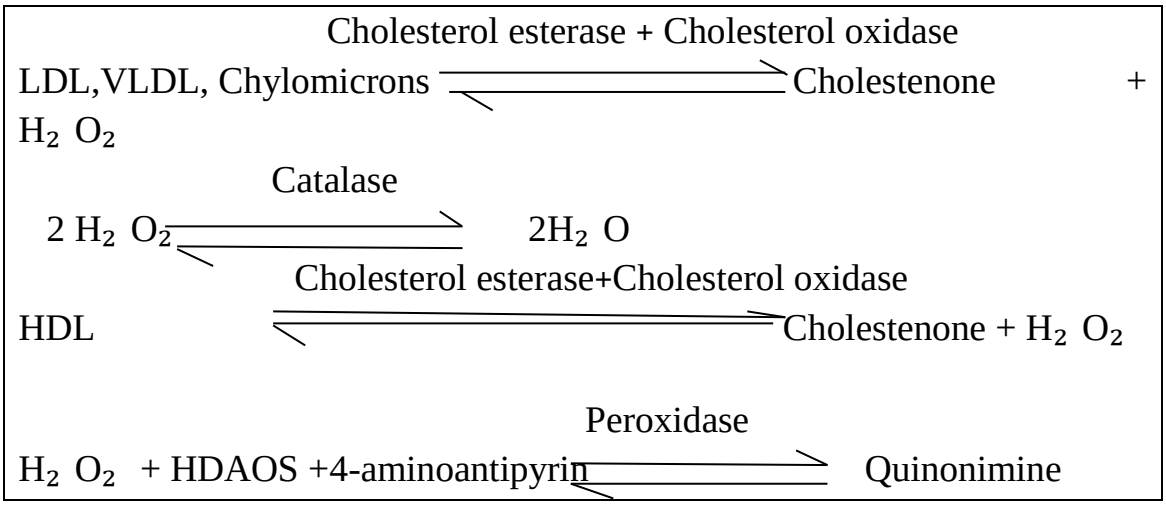


Figure (2-4) Measurement of High-Density Lipoprotein cholesterol concentration (Rifai N,*et al.*, 1997).

At 600nm, system tracks change in absorbance. The device uses absorbance change, which is directed proportional to the concentrate of

cholesterol in the sample and is used to calculate and express the HDL – cholesterol concentration .

Reagents

Table (2-7) Components with Concentration Reagents of High-Density Lipoprotein cholesterol Kit (Shenzhen Mindray Bio- Medical Electronics-Germany)

	Good's buffer	100mmol /L
	Cholesterol esterase	600U/L
R 1:	Cholesterol oxidase	380U/L
	Catalase	600U/L
	HDAOS	0.42mmol/L
	Good's buffer	100mmol /L
R2:	4-aminoantipyrine	1.0mmol/L
	Peroxidase	>2.8 U/mL
	Surfactant	<2%

2.2.2.3.2 Assay Procedure

Table (2-8) Assay procedure of High-Density Lipoprotein cholesterol Kit (Shenzhen Mindray Bio- Medical Electronics-Germany)

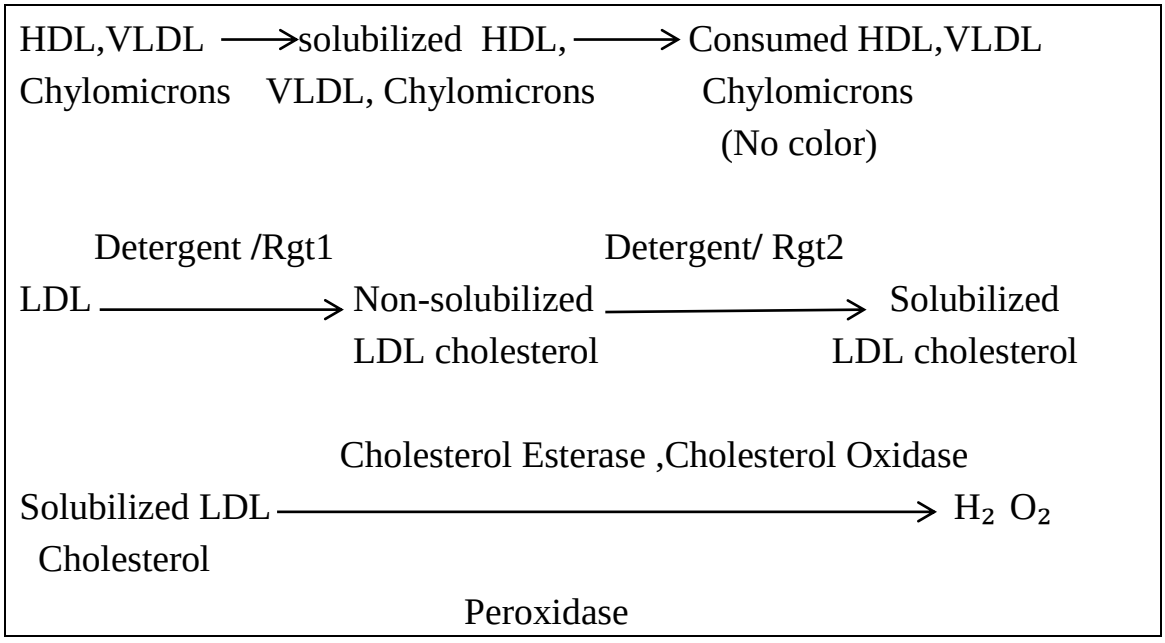
	Blank	Sample
Reagent 1	900µL	900 µL
Dist. water	12µL	_
Sample	_	12µL
Mixed and incubated for 5 min .at 37℃,then added:		
Reagent 2	300µL	300 µL
Mixed thoroughly, incubated at 37℃for 5 min., and then read the absorbance change value.		
ΔA= [ΔA sample]-[ΔA blank]		

2.2.2.4 Evaluation of Low-Density Lipoprotein cholesterol (LDL)

Level of LDL was estimated according to the manual procedure of kit Shenzhen Mindray Bio- Medical Electronics-Germany normal Low-Density Lipoprotein cholesterol ≤ 160.0 mg/dL (Rifai,*et al.*, 1999).

2.2.2.4.1 Principle of the Assay

the two-parts, liquid stable method for testing LDL-C levels directly in (serum) is the auto LDL™ cholesterol Reagent . The method based on special detergent properties that do away need for centrifugation steps or any treatment stage. (Reagent 1), is the detergent that solubilizes only the non-LDL lipoprotein particles. In a non-color forming reaction , The cholesterol released is consumed by cholesterol esterase and cholesterol oxidase. The remaining LDL particles soluble a second detergent (Reagent 2) , and a chromogenic coupler color form. Degree of color resulting from the enzymatic reaction with LDL-C is proportional to the level of cholesterol present in the specimen.



H_2O_2 +DSBmT +4-AA ⟶ color Development (Measured Bichromatically At 546 & 660nm)

Figure (2-5) Measurement of Low-Density Lipoprotein cholesterol concentration (Rifai N,*et al.*, 1997).

Reagents

Table (2-9) Components with Concentration Reagents of Low-Density Lipoprotein cholesterol Kit (Shenzhen Mindray Bio- Medical Electronics-Germany)

R 1:		
	Cholesterol esterase	600U/L
	Cholesterol oxidase	380U/L
	Peroxidase	>2.8U/mL
	4-aminoabtipyrine	1.0mmol/L
	Ascorbic acid oxidase	
	Preservative	
	Detergent1	
R2:		
		N,N-bis (4-sulfhobutyl)-
		m-Toluidine-disodium
		(DSBmT)
		Preservative
		Detergent 2

3.2.3.4.2 Assay Procedure

Table (2-10) Assay procedure of Low-Density Lipoprotein cholesterol Kit (Shenzhen Mindray Bio- Medical Electronics-Germany)

	Blank	Sample
Reagent 1	900µL	900 µL
Dist. water	12µL	_
Sample	_	12µL
Mixed and incubated for 5 min .at 37℃,then added:		

Reagent 2	300µL	300 µL
Mixed thoroughly, incubated at 37℃for 5 min., and then read the absorbance change value.		
ΔA= [ΔA sample]-[ΔA blank]		

2.2.8 Estimating the Levels of Some Immunological Parameters by ELISA Assay

2.2.8.1 Estimation of Interleukin–6 (Human,IL-6)

The IL-6 parameter level was calculated according to the an instrument procedure from Bioassay technology Laboratory- China. (Standard Range:2-600ng/L)

2.2.8.1.1 Principles :

The current experiment adopted the enzyme-linked immunosorbent assay method to determine interleukin-6 concentrations in the study population. The plates were coated with Anti-interleukin6 , followed by adding the samples to linked antibody by completely coated on the wells. The samples IL-6 is then bound by Biotinylated human IL-6 antibody. The biotinylated IL-6 antibody then binds to Streptavidin-HRP when added. After incubation, during the washing process streptavidin-HRP is eliminated. The substrate solution is then added , the color changes in direct proportion to the amount of human IL-6. The reaction is terminated by adding acid stopping-solution and the absorbance is measured under wavelingth 450 nm.

2.2.3.1.2 Evaluation Steps

1. All prepared solution, reagents materials and specimen warmed to normal environment temperature before use.
2. Choose some of strips that required for the work and inserted in the frames for use. Unneeded strips were stored between 2-8 °C.
3. 50 microliter of standard were added to well that detected for standard well.
4. A40µl from samples were added to other wells. 10 microliter Human-IL-6 antibody , and fifteen microliter streptavidin-HRP was added to wells samples and standard with mix processes . after these steps plates covered with a sealer and incubated for 60m. at 37°C.

5. Next step included remove sealer and washed plate 5 times by washing buffer. For every wash,300µ1 of wash buffer was soaked in wells from 30sec. to one minute. To washing automatically , aspirated method or decanted and washed for five times with washing buffer for each well. then plates were blotted with absorbent material such as paper towels or other.
6. substrate solution A, added to all wells then add 50 microliters substrate solution-B addition. Microplate was covered with a new sealer and incubated for ten minutes at 37 º in dark environment.
7. 50 microliter for final step to Stop reaction by stopping-solution addition to each well; immediate color was changed from blue to yellow .
8. An Optical density (OD) of every well was determined by a microplate reader within 10 m. under length wave 450 nm .

Standard curve of IL-6 was done using different concentration of IL-6 starts from (0.000, 100.00, 200.000,300.000 and 400.000) ng/L at 450 nm wavelength as illustrated in (fig.2.2)

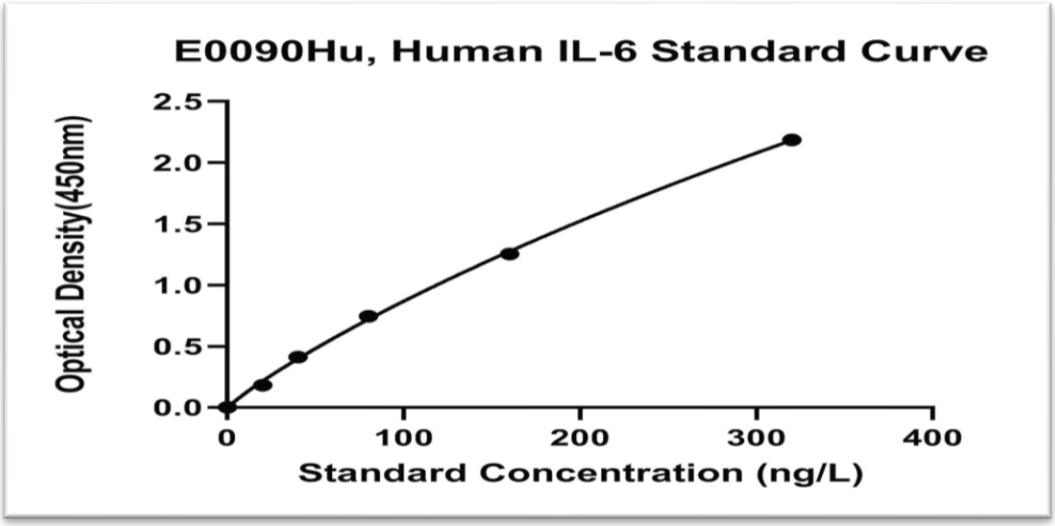


Figure (2.6): Standard Curve of IL-6
Kit for (Human) Interleukin 6ELISA-IL6 Bioassay technology Laboratory- China

2.2.8.2 Estimation of Human soluble Interleukin–6 Receptor (sILR-6)

The sIL-6R parameter level was calculated according to the an instrument procedure from Bioassay technology Laboratory- China. (Standard Range:0.5-150 ng/L)

2.2.3.2.1 Fundamental of the Assay

The current experiment adopted the enzyme-linked immunosorbent assay method to determine sIL-6R concentrations in the study population. The plates were coated with Anti- sIL-6R , followed by the step of adding the samples to linked antibody by completely coated on the wells. sIL-6R in the sample is subsequently bound by Biotinylated sIL-6R antibody. Streptavidin-Hours Redish Peroxidase is binds to the biotinylated sIL-6R antibody when adding. After incubation, streptavidin- Huors Redish Peroxidase is remove by the washing process. The sub-strate solution is then added and the color develops in proportion to the level of human sIL-6R. The reaction is terminated by adding acid stopping-solution and the absorbance is measured under wavelength 450 nano meter.

2.2.3.2.2 Assay Steps

Same the assay steps of IL-6

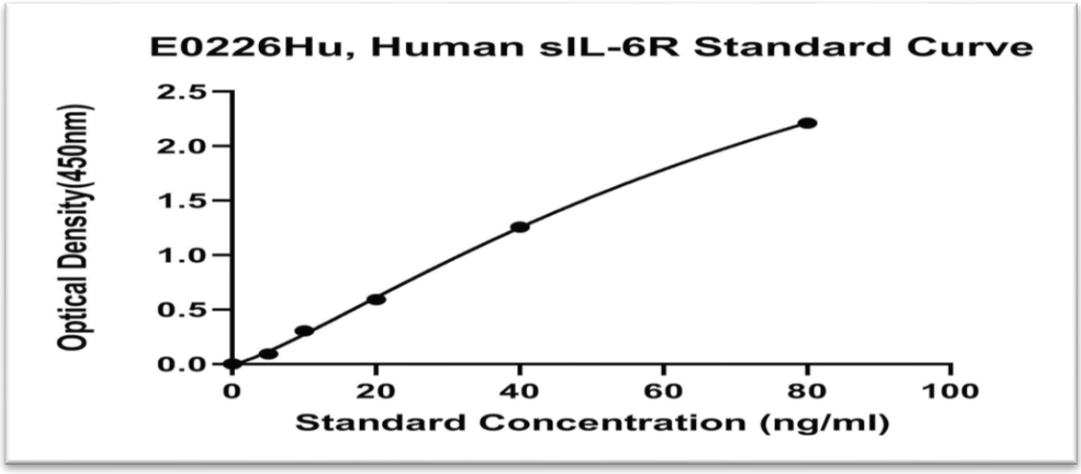


Figure (2.7): Standard Curve of sILR-6
Kit for Human soluble Interleukin–6 ReceptorELISA-sILR-6 Bioassay technology Laboratory- China

2.2.3.3 Human Tumor Necrosis Factor-alpha (TNF- α)

The level of TNF- α was estimated according to the procedure of kit manually by Bioassay Technology Laboratory- China.(standard Range:3-900ng/L)

2.2.3.3.1 Fundamental of the Assay

The current experiment adopted the enzyme-linked immunosorbent assay method to determine TNF-alpha concentrations in the study population. The plates were coated with Anti- TNF-alpha, followed by the step of adding the samples to linked antibody by completely wells coated . TNF-alpha in the specimen is subsequently bound by Biotinylated human-TNF-alpha antibody. Streptavidin-Horseradish Peroxidase is an enzyme then added and binds to the biotinylated TNF-alpha antibody. After incubation, streptavidin-HRP is removel during the washing process. The sub-strate solution is then added and the color develops in proportion to the human TNF-alpha concentrations. The reaction is terminated by adding acid stop solution and the absorbance is measured under 450 nanometer.

2.2.3.3.2 Assay Steps

Same the assay steps of IL-6

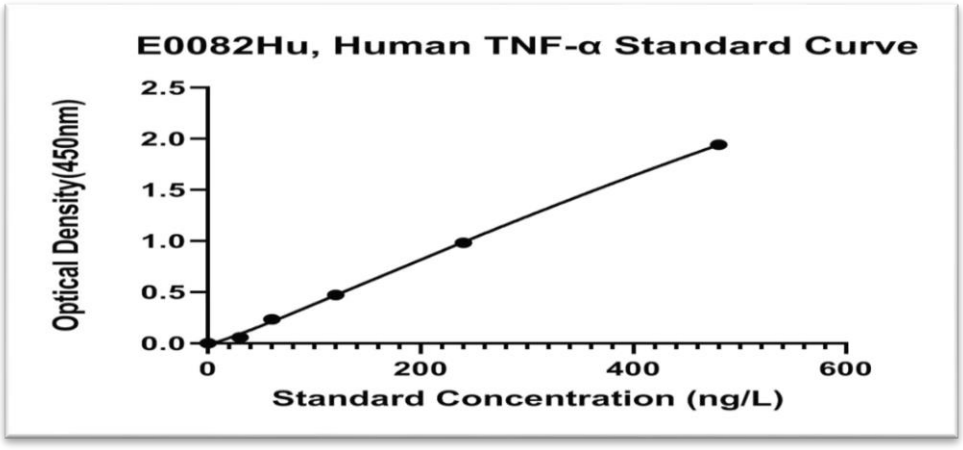


Figure (2.8): Standard Curve of TNF- α
Kit for Tumor Necrosis Factor-alpha -TNF-α Bioassay technology Laboratory- China.

2.2.4 Statistical analyses

Information from the questionnaire from all participants were entered in a data sheet and were assigned a serial identifier number. Multiple entry was used to avoid errors. The data analysis for this work was generated using The Statistical Package for the Social Sciences software, version 28.0 (IBM, SPSS, Chicago, Illinois, USA) and the Real Statistics Resource Pack software for Mac (Release 7.2) of the resource pack for Excel 2016. Descriptive statistics was performed on the participants' data of each group. Data was analyzed for means, and the standard deviation (SD) was computed for the continuous variables, whereas frequency was used for computing the qualitative data. The distribution of the data was checked using Shapiro-Wilk test as numerical means of assessing normality. The correlation between the analyzed parameters was estimated using Pearson regression and 95% Confidence Interval range which calculated by a non-conditional logistic regression. Significant differences in categorical variables among the parameters were confirmed through analytical statistical tests. A receiver operating characteristic (ROC) curve was analyzed to assess the research indicator for predicting IHD. The one-way analysis of variance (ANOVA) was done to compare the means of different groups. Results of all hypothesis tests with p-values ≤ 0.05 (two-sided) were considered to be statistically significant (Duncan *et al*., 1983).

Chapter Three
Results and Discussion

3. Results and Discussion

3.1 Demographic characteristics of Study Groups

Table (3-1) displays the descriptive data of the study population, as the table shows there is a significant increase (P≤0.05) in age mean of IHD cases compared with control group , values of BMI (0.001) were significantly increase (P≤0.05) in IHD cases compared with control, meanwhile males and females were distributed equally in all study groups. Regarding to blood pressure parameters, both systolic and diastolic blood pressures (0.000 ,0.000) were significant increased (P≤0.05) in IHD as compared with control subjects.

Table (3-1): The Demographic data of study subjects

Variable	**Mean ± SD**		P value
	Patients	**Control**	
Age (year)	60.4± 13.7	53.2± 9.6	0.001*
BMI (Kg/m^2)	26.85± 3.84	25.01± 2.21	0.04*
sex (Male/ Female) No. (%)	30/ 30	15/15	
Blood pressure	**Mean±SD**		
SBP(mmHg)	135.66	120	0.000*
DBP(mmHg)	88.5	80	0.000*
Family history of IHD	**NO. (%)**		
Yes	5/60	1/30	0.37^{NS}
No	55/55	29/30	
Smoking Status	**NO. (%)**		
Yes	18 (30%)	0	0.001*
No	42 (70%)	30 (100%)	

SD: Standard Deviation, NS: Non-Significant.
The mean difference is significant at the 0.05 level: *(P≤0.05), ^{NS} (P>0.05).

The statistical analysis showed that IHD patients had increasing mean of age as compared with control subjects, this finding of present study is logical, since increasing age considered as risk factor for

developing IHD, Similarly, older age was revealed as the significant predictors in IHD, which is corroborated by recent studies (Aggarwal *et al*., 2016; Sadiq and Hassan, 2017; Raffee *et al*., 2020a). The most likely explanation for the increased rates of IHD in older people is increase in prevalence of risk factors such as diabetes, obesity and hypertension in older age group. Current finding is consistent with the findings of a previous studies (Mohammed and Risun, 2020; Arrar and Al-Abedi, 2021), these studies indicated that most patients with IHD are within age 51 to 60 years old.

Obesity is an important conventional risk factors associated with adverse outcomes in the form of increased mortality and future acute coronary events (Liu *et al*., 2017). Numerous investigations reported that obesity may exacerbate IHD and increasing mortality rate even at young ages (Yang *et al*., 2014; Aggarwal *et al*., 2016). Also, the finding of present study agreed with another study conducted on Jourdan population revealed that IHD was high among patients with increasing BMI values (Raffee *et al*., 2020b).

According to analysis of IHD patient's data, it was found that their blood pressure parameters increased compared to healthy people. These results are consistent with the studies that found the majority of IHD patients had hypertension (Altaleb *et al*., 2017; Arrar and Al-Abedi, 2021).

Regarding to smoking, the current study agreed with other studies the prevalence of cigarettes smokers was 30%, which is higher than (28.0%) in men (Hammoudeh *et al.*, 2008), (21.0%) in women (Jabara *et al.*, 2007) and (16.1%) (Fakhrzadeh *et al.*, 2008), but less than (88.0%) in (Hadaegh *et al.*, 2009), and (57%) in (Jawameer and Saeed, 2021). However, the current study indicated that smoking in IHD patients not presented as a risk factors of CVD compared hypertension. This finding contradicts most previous studies (Senemar *et al.*, 2013; Mohammed *et al.*,

2018), and the reason may be because the small numbers of samples in current study.

3.2. Lipid profile biomarkers distribution in IHD cases and control subjects

Evaluation of (lipid profile levels) biomarkers distribution in the sera of the two study groups (IHD cases and control subjects) are shown in Table (3-2). The statistical analysis revealed significant increases ($P \leq 0.05$) in the TC, TG, and LDL, while there is a significant decrease ($P \leq 0.05$) in the sera HDL levels of patients comparing to control subjects.

Table (3-2): lipid profile levels in IHD cases and healthy group

Parameters	Mean ± SD		P value
	IHD	**Control**	
Lipid profile Parameter			
TC (mg/dl)	194.82 ± 59.39	166.08± 48.47	0.012*
TG (mg/dl)	187.55±73.11	137.89± 37.27	0.000*
HDL (mg/dl)	39.25 ±12.45	51.46±13.88	0.005*
LDL (mg/dl)	109.09±32.97	32.91±10.74	0.000*
TG/HDL	5.53±1.58	2.85±0.98	0.000*

SD: Standard Deviation, NS: Non-Significant.
The mean difference is significant at the 0.05 level: *($P \leq 0.05$), NS ($P > 0.05$).

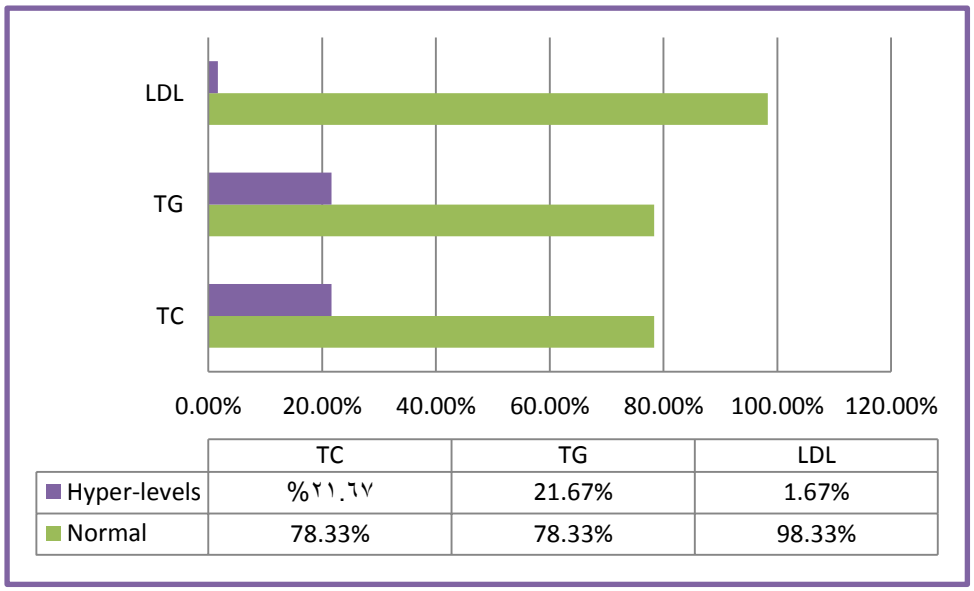
One of the fundamental risk factors for IHD is dyslipidemia, which attend from an excess in the levels of cholesterol, TG and LDL and a relief in HDL level in the serum of IHD patients (Jasim and Lefta, 2022).

The current study are consistent with findings of Assessment, Riezzo *et al.*, (2009) **.** who also recorded high levels of TC, TG, LDL, and VLDL and low levels of HDL-C; which are considered as one of the most common modifiable risk factors for IHD. A Similar findings also reported by Alam *et al* (2021). The key role of TC in CHD has given rise to the universally accepted cholesterol-diet-CHD hypothesis, According to this hypothesis,

boosted levels of plasma TC boost the risk of CHD, This risk reduced with decreasing TC levels, The Framingham study clearly demonstrated the association of elevated cholesterol with CHD (Alarabawy *et al*., 2016), The impact of the lipid profiles on the action of coronary arteries relies mostly on a several mechanisms of the physiological tasks of the lipid profiles.

Generally, atherosclerosis originate from LDL piling up on the inner wall of blood vessels, where apo-B(LDL) (a specific lipoprotein for atherogenic), supports aggregation of cholesterol in macrophages and thus an inflammatory responses in the inner wall of the vessel, the transfer of LDL from the bloodstream to the vessels walls trigger the onset for lipid deposition, These deposited lipids either returning to the blood and thus diminish the lipids deposited on blood vessels and thus diminish lesion development or oxidized by the action of reactive oxygene species or Leukocytes, or turn into foam cells having been devoured by macrophages (Kloc *et al*., 2020), ApoA-I(principal apolipoprotein of HDL, commonly it transports about 20% of total cholesterol in blood, and the moving of the additional cholesterol to the liver from the foam macrophages of arterial walls by mean of adenosine triphosphate-binding cassette transporters and by lecithin-cholesterol acyltransferase assistances transform the cholesterol esterification into complete HDL2 and HDL3 units after the pass of cholesterol from peripheral cells through adenosine triphosphate-binding cassette transporters (ABC), Transfer protein of cholesterol ester (CETP) transmissions to HDL cholesterol esters of additional apolipoprotein B- inclosing units, then, HDL units are stuck via hepatocytes through scavenger receptor B1 (Durrington, 2012; Deng *et al*., 2022).

The present study found to the relation between the hazard for IHD and depressed HDL levels. So, HDL is considered helpful for IHD health as it helps tear out cholesterol from the bloodstream, while LDL is considered injurious because it engages to the building up of cholesterol in the arteries (Chapman *et al*., 2004). The findings about all lipid profile of the current study are in accordance with previous studies (Laltesh and Ajay, 2018; Jasim and Lefta, 2022). Figure (3-1) displays the percentage of normal and hyper-levels of TC, TG, and LDL.



	TC	TG	LDL
Hyper-levels	%٢١.٦٧	21.67%	1.67%
Normal	78.33%	78.33%	98.33%

Figure (3-1): Percentage of Normal and Hyper-levels of TC, TG, and LDL in IHD patients.

The figure (3-2) displays the percentage of normal and hypo-levels of HDL in IHD patients.

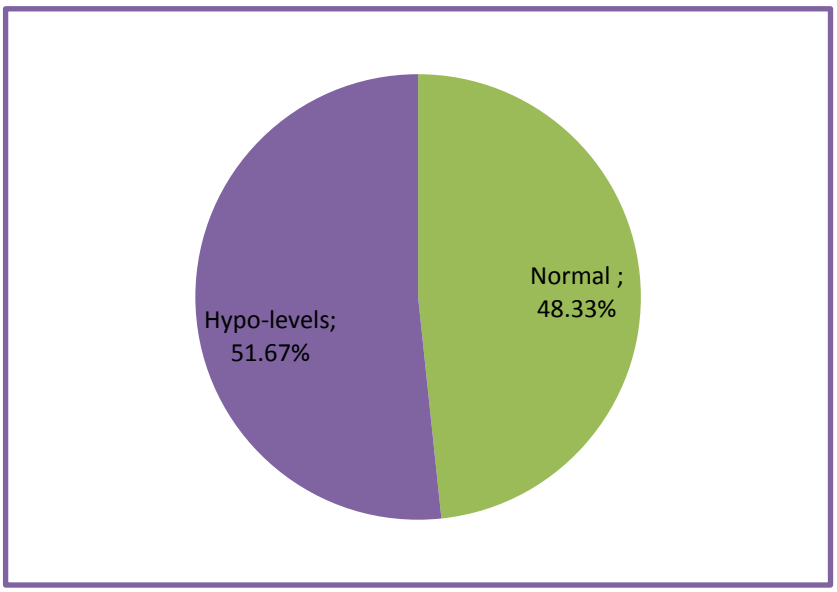


Figure (3-2): Percentage of Normal and Hypo-levels of HDL in IHD Patients.

3.3 Immunological biomarkers distribution in IHD cases and control subjects

Evaluation of immunological biomarkers (IL-6, sIL-6R and TNFα) distribution in the sera of the two study groups (IHD cases and control subjects) are shown in Table (3-3). , the results of the statistical analysis for immunological parameters showed a significant increase (P≤0.05) in patients comparing to control subjects.

Table (3-3): Some immunological biomarkers distribution in IHD cases and control subjects

Parameters	Mean ± SD		P value
	IHD	**Control**	
IL-6	1.83±0.21	1.42±0.29	0.001*
sIL-6R	27.82±9.77	12.69±2.98	0.003*
TNF-α	197.54±68.50	78.22±19.97	0.001*

SD: Standard Deviation, NS: Non-Significant.
The mean difference is significant at the 0.05 level: *(P≤0.05), NS (P>0.05).

In the current study the immunological parameters included in present study, as table (3-3) showed that are a significant increasing in IL-6 levels in IHD patients compared to healthy individuals. The current finding agree with another findings showed that there was a significant increase in the levels of IL-6 in IHD patients than in control group (Jabir *et al*., 2017). The results of the current study are also consistent with the results of a recent local study conducted on a sample of the Iraqi population (Muhi *et al*., 2023). Increasing levels of IL-6 could be attributed to the evolution and instability of atherosclerotic plaques through the activation of leukocyte and endothelial cells, or it could be explained as a result of various cytokines stimulation (Jabir *et al*., 2017).

IL-6 is known to play a role in the acute phase response, which is the body's immediate response to tissue injury, infection, or inflammation During this response, IL-6 stimulates the liver to produce acute phase proteins, such as C-reactive protein (CRP) and fibrinogen, CRP is a marker of inflammation and elevated levels are associated with an increased risk of IHD. Fibrinogen is involved in blood clotting, and elevated levels can increase blood viscosity and promote clot formation, contributing to the risk of IHD. Additionally, IL-6 can stimulate the proliferation and activity of platelets, which are involved in the formation of blood clots. This further contributes to the pro-thrombotic state associated with IHD. Also, IL-6 could stimulate monocytes both in an autocrine and paracrine manner. These events contribute in the deposition of fibrinogen in blood vessel wall, and a consequence atherosclerosis development, which is a hallmark of IHD. As well as, IL-6 stimulates the hypothalamic-pituitary-adrenal axis (Tomas *et al*., 2015; Muhi *et al*., 2023).

In regard to sIL-6R, current study showed independent association with risk of IHD. Indeed, the observation that sIL-6R increased in IHD patients highlights the important role of IL-6 in IHD, Based on calculation

of intramolecular affinities it was originally assumed that most circulating IL-6 was in a complex with sIL-6R (Mani *et al*., 1999). The result of present study supported with a previous investigation (Velásquez *et al*., 2015) which expected that as IL-6 transactivation is involved in IHD pathogenesis, it would be expected that sIL-6R would also implicated with it. Our results provide further support for findings from Mendelian randomization studies suggesting that IL-6R signaling seems to have a causal role in the development of CAD (Interleukin-6 Receptor Mendelian Randomization Analysis (IL6R MR) (Consortium, 2012).

On the other hand, the results of the current study disagree with other studies that showed that only a minor fraction of IL-6 is bound to sIL-6R, and only the level of IL-6 provides a better reflection of CVD risk than that of sIL-6R and may imply that classical IL-6 activation plays a more important role in cardiovascular disease than transactivation (Baran *et al*., 2018; Edsfeldt *et al*., 2023).

On the basis of the results of current study, a significant increase in TNF-α level in IHD as compared with control, TNF-α is considered a pleiotropic cytokine that influence several activities of cellular system, It affects the lipid metabolism, coagulation endothelial task, along with insulin resistance (Tronchon *et al*., 2008). Chronic inflammatory events are labeled by uplifted circulatory levels of pro-inflammatory cytokines, adhesion molecules, as well as cytokine-responsive acute-phase proteins, Several of inflammatory indicators had been singled out to presage future cardiovascular risk in acute coronary syndrome patients. TNF-α levels in the blood have been linked to atherosclerosis of coronary artery risk factors such as dyslipidemia, overweight, and inflammation (Gustafson, 2010). In a sample of stable and unstable coronary patients, TNF-α was significantly associated with the gravity of coronary disease; this impact could be a signal to chronic inflammatory load and a risk factor for serious coronary

diseases (Gotsman *et al*., 2008). The results of the current study are consistent with the study of (Abdulfattah and Samawi, 2023) who indicated an increase in TNF-α in IHD patients with and without angina pectoris.

3.4 Level of the age on studied biomarkers

Distribution of study population according to their age is shown in figure (3-3). They were divided into three age categories: (≤50, 51-60 and ≥ 60), the highest percentage of the IHD patients and control was within age category ≥ 60 years, while the lowest percentage was (20% and 26.70%) within age category (51-60y) respectively.

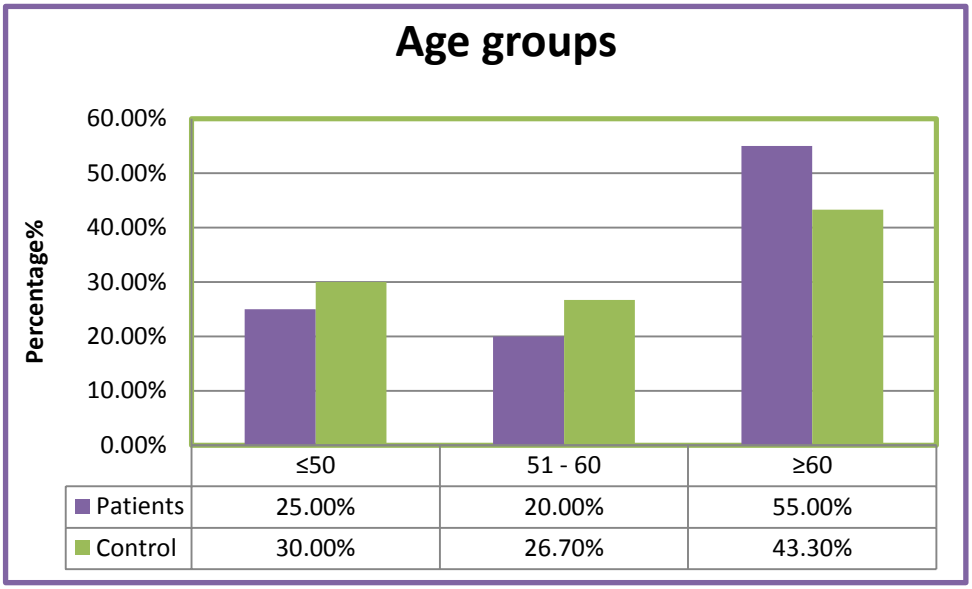


Figure (3-3): Distribution of the IHD Patients and Control According to the Age

Age is among the most important risk factors for predicting incident cardiovascular disease, Based on previous experience studies in the United States the average risk of developing cardiovascular disease for a 30-34 years old male is 3%, this number raises some seven fold to 21% for a comparable individual aged 60-64 years (Wilson *et al*., 1998). Present investigation agrees with previous ones which indicated that incidence of

IHD mounting from age 40 years in both Sexes while the incidence is low in individuals of less than 40 years old (Aiyer *et al*., 2007; Sadiq and Hassan, 2017).

The exact importance of age-related risk compared with other cardiovascular disease risk factors illustrated by the Framingham Heart Study (To get a better knowledge of the epidemiology of coronary heart disease (CAD) in the United States, the Framingham Heart Study (FHS) was founded in 1948. Major risk factors for congestive heart failure (CAD) were established in 1961 by seminal work (high blood pressure, high cholesterol, and left ventricular hypertrophy on the ECG). These variables eventually served as the foundation for multivariable 10-year and 30-year risk-prediction algorithms) (Andersson *al et.*,2019) , that has resulted in a 14-point scoring system to predict incident 10-year cardiovascular disease, In this system, the increasing risk characterized by a higher score, up to 7 points can be attributed to age alone (Stocker *et al*., 2004). In addition, the cumulative risk for IHD in males by age 70 years is 35% and by age 90 years is 49%, While the women typically develop IHD about 10 years later than men with a cumulative risk of 24% and 32% by age 70 and 90 years, respectively, On the other hand, the subjects with a family history with atherosclerosis are at the utmost risk for developing IHD even though in early ages (Scheuner, 2003).

3.4.1 Level of Age on lipid Profile and Immunological Biomarkers in IHD Patients

The effect of age on the studied criteria is shown in Table (3-4). Current study found that TG and LDL were a significant (P≤0.05) increased by increasing age. The highest levels of TG and LDL were in age categories (51-60y) and (≥ 60), which was significantly (P≤0.05) highest than (≤50) age category. On the other hand, the remaining lipid and immunological biomarkers showed non-significant (P>0.05) differences among age categories.

Table (3-4): Level of age on lipid profile & immunological biomarkers in IHD Patients

Parameters	Age groups (Mean ± SD)			P value
	≤50	**51-60**	**≥ 60**	
Lipid profile parameters				
TC (mg/dl)	163.6 ± 3846 **b**	206.76±63.68 **a**	204.78 ±62.82 **a**	0.002*
TG (mg/dl)	187.92±51.30 **a**	213.54±18.68 **a**	234.13±38.11 **a**	0.71NS
HDL (mg/dl)	41.66±8.96 **a**	37.51±10.01 **a**	38.87±14.02 **a**	0.282 NS
LDL (mg/dl)	99.39±28.81 **b**	93.86±38.77 **b**	117.52±30.05 **a**	0.01*
TG/HDL	4.69±1.47 **a**	6.14±2.70 **a**	5.77±1.92 **a**	0.225 NS

Parameters	Age groups (Mean ± SD)			P value
	≤50	**51-60**	**≥ 60**	
Immunological parameters				
IL-6	1.87±0.24 **a**	1.86±0.22 **a**	1.81±0.20 **a**	0.600 NS
sIL-6R	31.20±12.47 **a**	32.45±14.33 **a**	28.72±13.37 **a**	0.786 NS
TNF-α	200.73±83.04 **a**	229.11±71.02 **a**	184.61±57.88 **a**	0.145 NS

SD: Standard Deviation, NS: Non-Significant
Different small letters refer to significant differences within-patients comparison, similar letters refer to non-significant differences
***significant (P≤0.05), NS (P>0.05)**

The current study showed a significant increase in the levels of TC and LDL in the older age groups, which had the highest incidence of ischemic heart disease. This finding is logical and justified because cholesterol and other lipid profile are considered among the most important risk factors for heart disease. IHD appears in any age when risk factors to atherosclerosis are present, such as smoking, Hypertension, diabetic mellitus, genetic hypercholesterolemia and other causes of lipidemia, this agreement goes with report that proved age, blood lipid level (TC, HDL and, triglyceride) are significant increase in patients with IHD (Aiyer *et al*., 2007; Kotseva *et al*., 2009).

Previous studies have indicated the importance of the effect of age on lipid profile as they are inevitable risk factors for the development of ischemic heart disease. A study by Sadiq and Hassan (2017) revealed a significant relation between cholesterol and HDL among age range (50-59), (60-69) and (70-79) years.

As for the IL-6, sIL-6R, TNF-α, age did not show any significant effect on the distribution of these factors between age groups, because these cytokines are each related with IHD risk autonomous of conventional risk factors and in an approximately log-linear manner; this findings support to the inflammation hypothesis in heart disease (Kaptoge *et al*., 2014); as well a further investigations are required to estimate causality.

3.5 Level of patient's BMI on studied biomarkers

Both study groups were subdivided according to body mass index (BMI) into three groups (Normal weight (18.5-24.9), Overweight (25-29.9) and Obese (≥ 30) as shown by Figure (3-4); the majority of IHD patients and control (40%, 60%) were normal weight, while (36.67%, 30%) of them were overweight and (23.33%, 10%) was obese, respectively.

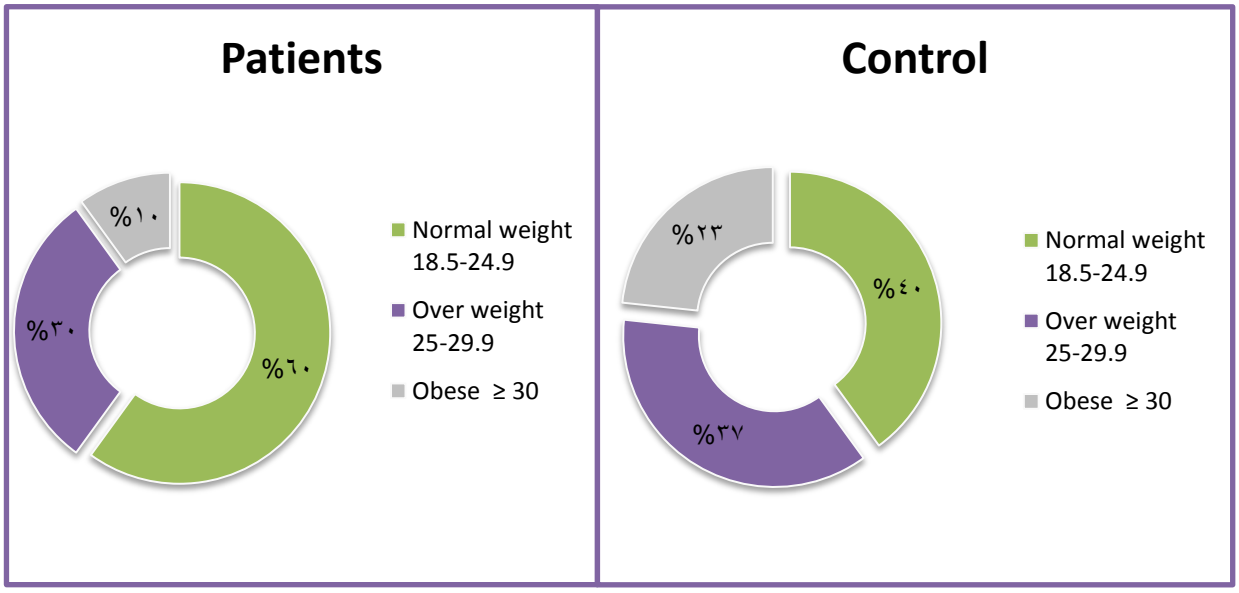


Figure (3-4): Subgroups of BMI According to the Traditional World Health Organization (WHO) (normal weight range is 18.5-24.9) Kg/M², overweight range is (25-29.9) Kg/M² , obese is ≥ 30 Kg/M² (*World Health Organization*,2009).

Body mass index is an essential index of measurement for linking a person's body weight to their height. BMI had been applied by WHO as the standard for recording obesity statistics (Eknoyan, 2008). Although it does not accurately quantify the percentage of body fat, but it is a functional tool to assess a healthy body weight relayed on how tall an individual. Due to its simplicity of measurement as well as calculation, BMI is the most widely used diagnostic tool to identify weight problem in medical research (Jawameer and Saeed, 2021).

Current analysis revealed that the prevalence of IHD was mainly in normal-weight and over-weight and the present of obesity were in IHD 10% lower than in control subjects (23%). Nowadays, also for control subjects, the overweight and obesity status represent a crucial public health problem and nutritional disturbance that can be assigned to interrelated factors including consumption of high-energy (low-nutrient) diet and fall off physical activity (Baikpour *et al*., 2017).

The reason for the low percentage of obesity in patients can be attributed to the small number of samples included in the current study due to the limited study time. It can also be justified on the basis that patients, after suffering from heart disease, tend to follow a diet to reduce their weight in order to obtain an ideal body weight to reduce the risk of IHD; On the other hand, a significant number of patients suffer from weight gain, as the figure (3-4) shows.

Obesity is one of the most common risk factors for atherosclerosis, IHD, and diabetes mellitus (Ezzati *et al*., 2002; Sarwar *et al*., 2007). It is associated with many of the known cardiovascular risk factors, such as hypertension and dyslipidemia (Pi- Sunyer, 2002). Current findings appropriate with a previous investigations that had revealed that in patients with heart failure, acute coronary syndromes (Wienbergen *et al*., 2008; Dhoot *et al*., 2013) atrial fibrillation (Sandhu *et al*., 2016), or after coronary revascularization (Gruberg *et al*., 2002), there may be an "overweight paradox" phenomenon, with better cardiovascular prognosis among patients with higher BMI (Lavie *et al*., 2016; Sandhu *et al*., 2016). In a recent research on elderly patients >80 years, were undergoing percutaneous coronary intervention, mortality was highest in the lowest BMI tertile in acute coronary syndromes, but this association was not confirmed in patients with stable CHD (Leistner *et al*., 2019). Indeed,

association between BMI and IHD outcomes seems maladjusted and complex; so further researches are required to explain this association.

3.5.1 Level of BMI on lipid Profile and Immunological Biomarkers in IHD Patients

Table (3-5) summarizes the effect of BMI on studied biomarkers in IHD patients. From all lipid biomarkers, only TC showed a significant (P≤0.05) differences among BMI groups of IHD patients, the highest levels of TC were in overweigh and obese groups, while the lowest level found in normal weigh group. From immunological biomarkers, a significant (P≤0.05) increased was found in levels of sIL-6R in obese patients while TNF-α showed a significant (P≤0.05) increased in overweight and obese groups compared with normal weight group.

Table (3-5): Level of BMI on lipid Profile Immunological & Biomarkers in IHD Patients according to BMI group

Parameters	BMI groups (Mean ± SD)			P value
	Normal weight	**Overweight**	**Obese**	
Lipid profile parameters				
TC (mg/dl)	166.23±33.29 [b]	194.51±59.58 [a]	214.48±62.83 [a]	0.008 [*]
TG (mg/dl)	164.6±36.65 [a]	173.69±69.03 [a]	195.42±91.13 [a]	0.326 [NS]
HDL (mg/dl)	40.42±13.43 [a]	37.44±13.18 [a]	40.27±9.39 [a]	0.381 [NS]
LDL (mg/dl)	110.51±22.97 [a]	112.39±37.72 [a]	127.13±12.84 [a]	0.118 [NS]
TG/HDL	5.82±2.40 [a]	5.81±1.91 [a]	5.12±2.07 [a]	0.557 [NS]

Parameters	BMI groups (Mean ± SD)			P value
	Normal weight	**Overweight**	**Obese**	
IL-6	1.79±0.12 [a]	1.84±0.25 [a]	1.85±0.21 [a]	0.441 [NS]
sIL-6R	26.6±25.66 [b]	29.72±14.49 [b]	38.05±12.73 [a]	0.011 [*]
TNF-α	174.31±59.89 [b]	198.17±77.56 [a]	223.32±50.75 [a]	0.020 [*]

SD: Standard Deviation, NS: Non-Significant
Different small letters refer to significant differences within-patients comparison, similar letters refer to non-significant differences
[*]significant (P≤0.05), NS (P>0.05)

The subgroups analysis of IHD patients according to BMI categories regarding cardiovascular outcomes, did show a significant impact only in TC levels, they significant increase in obese and over-weight IHD patients. As for the rest of the lipid profile, there was no significant difference, which indicates that all patients, regardless of the nature of their bodies, had high levels of lipid profile, which reinforces the importance of these biomarkers in the development of IHD disease.

It has been observed that many lipid/lipoprotein abnormalities are prevalent in obesity and heart problems, collectively termed as dyslipidemia, however, these dyslipidemias are often hyperlipidemia wherein majority of lipids are shifted towards the upper limits of range or higher than the range (Shabana *et al*., 2020). The results of present study compatible with a previous study El-Mikkawy *et al*., (2020) that also recorded a significant differences in subjects with overweight and different grades of obesity in TC levels, while insignificant differences were reported in serum HDL. Also, our results agree with Ashcheulova *et al*., (2018) study in context of the significant differences for TC, and insignificant differences for TG and LDL.

Obesity is a key feature of metabolic syndrome, and the linkage between them has been attributed to the status of chronic inflammatory state (Stępień *et al*., 2014). Despite IL-6 levels were increased in overweight and obese patients, but the results of statistical analysis showed insignificant differences, while sIL-6R and TNF-α showed a significant increase in overweight and obese patients. In this context, Kaptoge *et al* (2014) revealed a statistically significant correlation between IL-6 and BMI. Present finding match previously published study (El-Mikkawy *et al*., 2020), which investigated the relationship between IL-6 and obesity. In obesity, macronutrients excess in adipose tissues trigger the release of

inflammatory cytokines such as IL-6, TNF-α, leading to chronic inflammation in obese (Ellulu *et al*., 2017).

Comparable findings were previously reported in a study of Baikpour *et al* (2017) They recorded a significantly positive correlation was found between serum levels of TNF-α and BMI. A similar finding conducted by Khaodhiar *et al*. (2004) indicated that obese subjects had significantly higher serum levels of TNF-α, IL-6, and CRP. Moreover, weight loss was associated with reduced of plasma levels of TNF-α and IL-6 by 25–30% (Bruun *et al*., 2003). There is a close relationship between metabolic pathways and inflammation, Macrophages and adipocytes are directly involved in the production of adipocytokines family members including IL-1, IL6, IL8, IL-12, interferon, TNF-α, and resistin (Frühbeck, 2004).

The death of adipocytes is very common in obese individuals and has been attributed to adipocyte hypoxia secondary to adipose tissue expansion, Adipocyte hypoxia may actively participate in the development of obesity-related inflammation by increasing adipocytokine production and promoting the expression of pro-inflammatory genes (Ye *et al*., 2007).

Regarding to sIL-6R result, the present data consistent with a previous study that proved a boosting in receptor of IL-6 expression in hypothalamus, suggesting a possible role for IL-6 in controlling appetite and energy intake (Stenlöf *et al*., 2003). Circulating levels of IL-6 were found to be increased with increasing adiposity and to be associated with the development of obesity-related complications such as cardiovascular diseases (Lowe *et al*., 2014).

3.6 Level of the patient's sex on studied Biomarkers

According to the current study, the male and female samples were equal in both patients (30 males and 30 females) and controls subjects (15 males and 15 females), as shown in Figure (3-5).

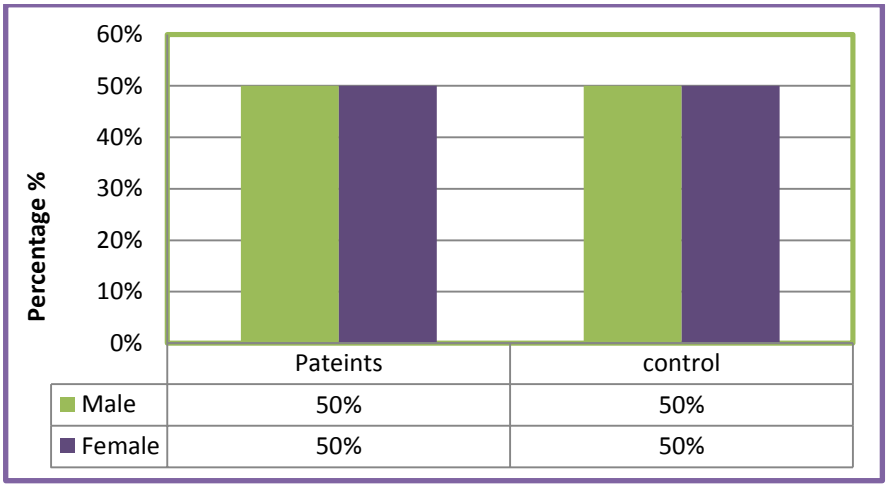


Figure (3-5): Distribution of the Study Groups According to Sex

3.6.1 Level of sex on lipid profile and immunological biomarkers in IHD Patients

The effect of sex on lipid profile biomarkers is shown in the Figure (3-6), where the results of the statistical analysis showed only a significant increase ($P \leq 0.05$) in levels of LDL in female compared with male in IHD patients only, while non-significant differences ($P > 0.05$) were recorded in the remaining lipid biomarkers. It is worth noting that the entire lipid biomarkers did not show any significant differences between males and females in the control group.

Regarding to immunological biomarkers, sex did not show any significant ($P > 0.05$) effect on the levels of the immunological biomarkers (IL-6, sIL-6R and TNF-α) included in the study in both IHD patients and healthy control subjects alike, as illustrated in Figure (3-7).

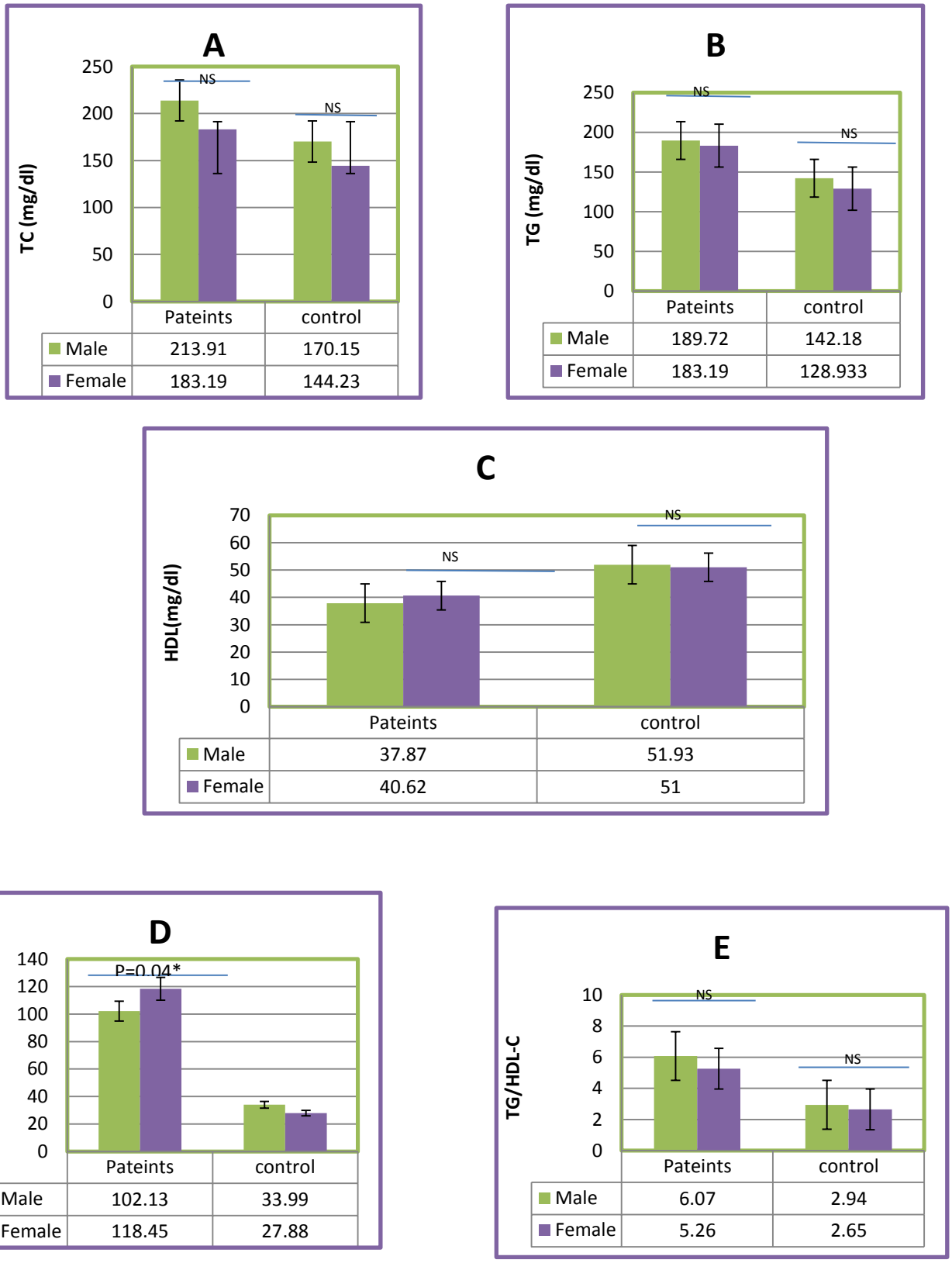


Figure (3-6): Level of Sex on lipid Profile Biomarkers in IHD Patients and Control Subjects (A) TC, (B) TG, (C) HDL, (D) LDL, and (E) TG/HDL-C ratio

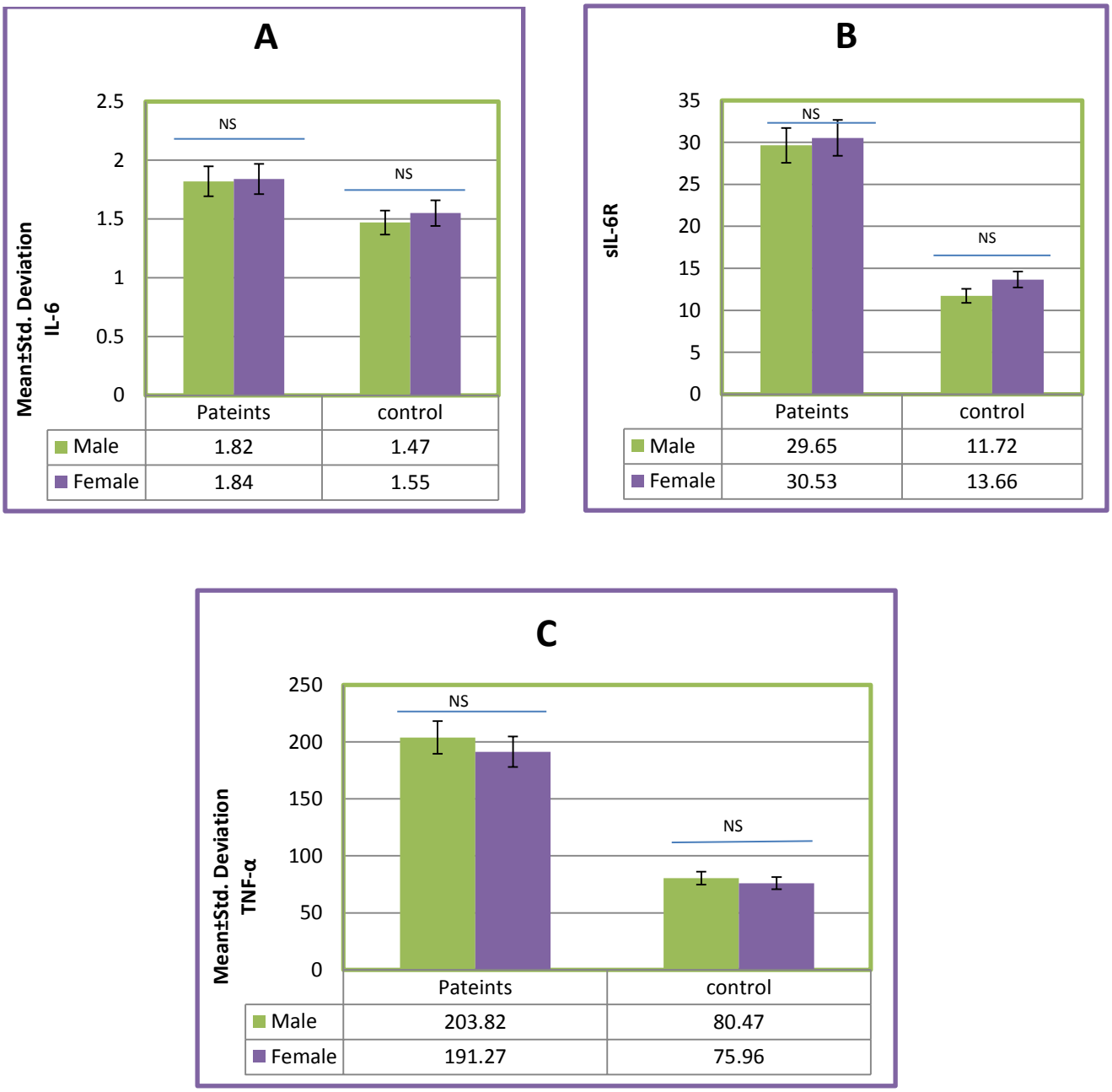


Figure (3-7): Level of Sex on Immunological Biomarkers in IHD Patients and Control Subjects (A) IL-6, (B) sIL-6R, and (C) TNF-α

According to the results of the current study, it was found that most of the factors studied did not show any significant difference between males and females with ischemic heart disease. This result may be due to the fact that most of the patients subject to the study are elderly, meaning that the differences between them are almost non-existent due to due to menopause in females participants in the study and the absence of hormonal effects that could constitute a difference between male and female; as well as the general aging process might contribute to this lack of distinction between males and females in the context of metabolic imbalances or immune output. It's essential to consider these changes when interpreting research results, especially when studying conditions that may be affected by Sex-specific factors like cardiovascular health. These findings are supported by the study conducting by Shabana *et al*. (2020) that also demonstrated insignificant differences between male and female with CHD in context of TC, TG, and HDL

In contrast to the results of the current study, the study conducted by Zegeye *et al*. (2021) showed that IL-6 as well as sIL-6R were significantly increase in males with Myocardial infarction compared to females patients. This difference from previous studies may be due to the small sample size in addition to the difference in techniques used to evaluate the factors included in the study.

3.8 Distribution of study population According to blood pressure

According to blood pressure, both study population (IHD patients and control subjects) were divided into two groups (normal and hypertension), as shown in Figure (3-8). The figure shows that all subjects of the control group were in the normal blood pressure group (100%), while most of the IHD patients, (74%) were in hypertension group and (26 %) were in the normal blood pressure group.

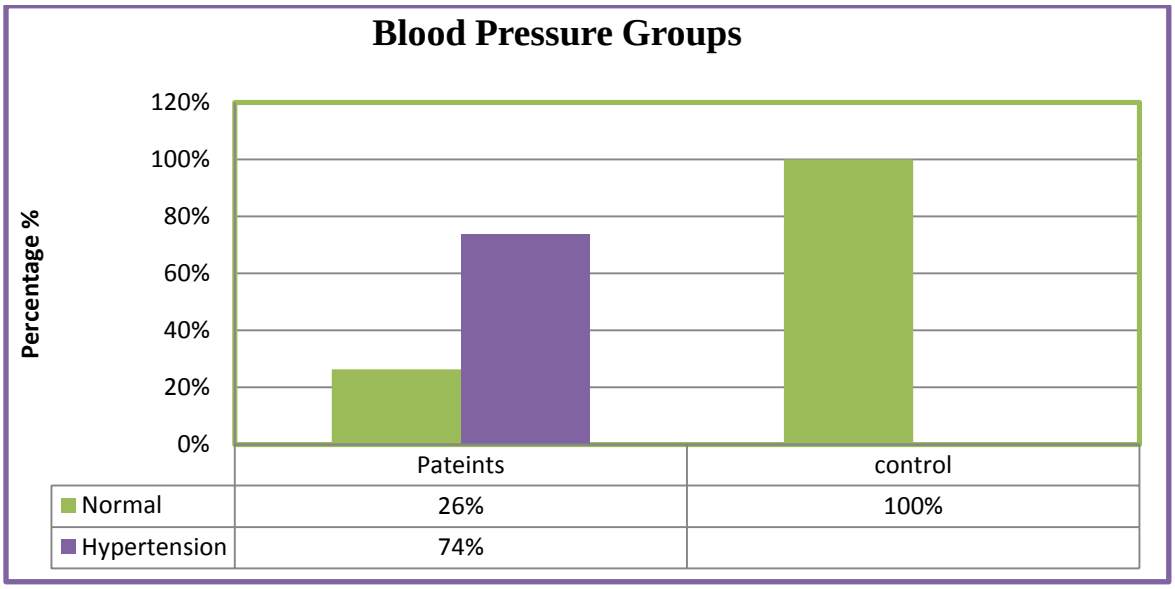


Figure (3-8): Distribution of the Study Groups According to Blood Pressure Status

Generally, hypertension duplicates the risk of heart disease and significantly accelerates the development of atherosclerosis (Špinar and Vítovec, 2003). Blood pressure plays a crucial role in the atherosclerotic process. The risk of cardiovascular complications increases continually along with the blood pressure, starting as low as from the 110/70 mmHg level. Systolic blood pressure is more predictive of mortality, especially in the elderly who most often suffer from isolated systolic hypertension. Furthermore, the combination impacts between hypertension and dyslipidemia is very common in IHD (Špinar, 2012).

Hypertension is established risk factors that trigger initiation and progression of atherosclerosis, the underlying mechanism for most of CVDs including IHD (Rafieian-Kopaei *et al*., 2014).

In the current study, the percentage of IHD suffering from high blood pressure is nearly similar to a previous study results in Sulaymaniyah (Mohamad *et al*., 2004). Also, the results of the current study are compatible to the results of Raffee *et al* (2020), as they showed that hypertension was possible risk factors among IHD young Jordanian population.

Hypertension is a leading cause of cardiovascular events, which contributes greatly to mortality and disability. With the increased understanding of immunology, evidence that the immune system may lead to hypertension is increasing (Drummond *et al*., 2019). It has been proved to induce IL-6 and intensify the endothelial dysfunction herewith triggering atherogenesis (Loperena *et al*., 2018). In addition, treatment of hypertension in has been shown to reduce IL-6 production (Vazquez-Oliva *et al*., 2005); so, it is logical that elevation in IL-6 may mediate the partially impact of smoking and hypertension in the course of atherogenesis. It is also tempting to speculate that smokers and hypertensives could benefit more from IL-6 blocking interventions (Zegeye *et al*., 2021). The inhibition of IL-6 attenuates the development of salt-sensitive hypertension in rat models. The deletion of IL-6 can prevent the activity of the JAK2/STAT3 pathway, which plays a role in Ang II-induced hypertension (Hashmat *et al*., 2016). The circulating levels of IL-6 have a positive relationship with blood pressure (Chae *et al*., 2001). Clinical data indicates that the hypomethylation of the IL-6 gene promoter may increase the risk of essential hypertension by upregulating the expression of IL-6 (Mao *et al*., 2017).

Chronic inflammation has been shown to exacerbate atherosclerosis, thus IHD mainly by dictating the thickness of the fibrous cap and vulnerability of the plaque by altering the balance between extracellular matrix production and degradation (Hansson *et al*., 2015).

3-9 Correlation analysis

3-9-1 Correlation among lipid Profile and immunological biomarkers for IHD Patients

Table (3-6) illustrates the correlation analysis among lipid profile and immunological biomarkers for IHD Patients. Height showed a significant positive correlation with weight. TG had a significant positive correlation with TC. HDL had significant invers correlation with Height and TC. LDL showed a significant positive correlation with TC and TG. Finally, sIL-6R had a significant positive correlation with IL-6. The remaining variables showed in significant correlation.

Table (3-6): Correlation among lipid profile and immunological biomarkers for IHD Patients.

Parameters	Value	Age	Weight	Height	TC	TG	HDL	LDL	IL-6	sIL-6R	TNF-a
Age	R.	1.000	-0.220	-0.025	-0.097	0.008	-0.118	0.025	-0.039	-0.029	-0.038
	P.		0.091	0.851	0.459	0.949	0.369	0.852	0.769	0.828	0.774
Weight	R.		1.000	0.337**	0.150	0.192	-0.105	0.199	-0.133	0.017	0.081
	P.			0.008	0.254	0.142	0.424	0.128	0.310	0.897	0.538
Height	R.			1.000	-0.115	0.067	-0.278*	0.058	-0.159	-0.098	-0.125
	P.				0.380	0.610	0.032	0.658	0.225	0.458	0.341
TC (mg/dL)	R.				1.000	0.395**	-0.298*	0.355**	0.049	0.073	0.236
	P.					0.002	0.021	0.005	0.711	0.579	0.070
TG (mg/dL)	R.					1.000	-0.093	0.790**	-0.186	-0.115	0.143
	P.						0.482	0.000	0.155	0.382	0.276
HDL (mg/dL)	R.						1.000	-0.091	0.088	0.213	0.187
	P.							0.488	0.503	0.102	0.152
LDL (mg/dL)	R.							1.000	-0.067	-0.012	0.158
	P.								0.611	0.926	0.228
IL-6 ng/ml	R.								1.000	0.447**	0.019
	P.									0.000	0.884
sIL-6R ng/ml	R.									1.000	0.121
	P.										0.357

-Pearson and Spearman's correlations were performed to assess the association strength and direction between the two continuous variables.
*. Correlation is significant at the 0.05 level.
**. Correlation is significant at the 0.01 level.

The current study addresses the patterns of interrelation between the studied factors to demonstrate their role in ischemic heart disease. The correlation analysis revealed some of these interrelations; where the Height showed a significant positive relation with weight; weight and height are correlated elements that used to calculate the BMI values, which works on the premise that weight increases proportionately to height squared, so that dividing weight by height squared results

in an index that is uncorrelated with height. This is an attractive quality for researchers and clinicians who want to analyze or assess body weight while accounting for the fact that taller people are generally heavier and shorter people are generally lighter (Johnson *et al*., 2020). So, it is logical to found a positive correlation between them.

TG had a significant positive correlation with TC. In previous study, it was reported that there was a positive correlation between TC and TG (Fan and Liu, 1995). The multiple linear regression equation for TC and TG had been established in some diseases (Ma *et al*., 2017; Zhang *et al*., 2020).

HDL had significant invers correlation with Height and TC; the finding of current study is consistent with a previous study that reported a significant inverse correlation between BMI and HDL and insignificant correlation between BMI and LDL was reported in other disease like diabetes (Hussain *et al*., 2019). These results are important to indicate that there is modest impact of BMI and its elements (height and weight) on lipid profile, Therefore, assessment and management for altered blood lipids should not be based on a patient's body weight or height; nor BMI. Regarding relation with TC, the present finding in line with study of (Miller *et al*., 2007), which reported an inverse negative correlation for HDL with TC and TG. On the other hand, current study disagrees with Hussain *et al*., (2019) who reported a positive correlation between HDL and TC. Moreover, LDL showed a significant positive correlation with TC and TG. The present study is in line with a previous investigation which also proved the existence of a significant positive correlation for LDL with each of TC and TG (Duan et al., 2020). LDL is often accompanied by increased TG, ApoB and decreased HDL levels (Kwon *et al*., 2006). Multiple linear regressions also showed that in CAD and metabolic syndrome patients, TG and TC were the most important

determinants of serum LDL concentrations (Yazdandoust *et al*., 2012), Consequently, correlation between LDL and serum lipids was analyzed. As a result, there is a significant positive correlation between LDL levels and TC and TG, which is consistent with findings reported by other groups and these associations may reflect the synergistic effect of lipid disorders in patients with ischemic heart disease.

Generally, various genetics, lifestyles, and environmental factors could all help in accelerate the cholesterol deposition to cause different heart disorders. Traditionally the ultimate villain in this interplay had always been the (low density lipoprotein cholesterol) LDL (Taskinen *et al*., 2003). Dyslipidemia is a contributing factor for IHD. It was proven that hyperlipidemia reduces vascular endothelial function (Duan *et al*., 2000).

The present finding about positive correlation between IL-6 and it soluble receptor agrees with study of (Zhou *et al*., 2020). Circulating IL-6 and the soluble form of the membrane bound alpha IL-6 receptor (sIL-6Rα) can form the heterodimer IL-6: sIL-6Rα (hereafter termed "B" for binary complex), which binds directly to the ubiquitously expressed membrane bound beta IL-6 receptor, also called glycoprotein 130 (gp130), and transduces a pro-inflammatory cascade known as the trans-signaling pathway (Morieri *et al*., 2017). There is ample evidence to support the potential involvement of IL-6-trans signaling in the pathogenesis of atherosclerosis. A zoological study has shown that hepatic-specific gp130-/- mice are resistant to atherosclerosis, probably owing to macrophage infiltration of atherosclerotic plaques and reduction of acute phase protein levels (Luchtefeld *et al*., 2007). Another study has identified that the IL-6 trans-signaling pathway plays a critical role in maintaining chronic inflammation in atherosclerosis (Hartman and Frishman, 2014).

On the other hand, the results of the current study differ from previous studies that showed no significant correlation between IL-6 and its receptor in other diseases (Bal and Laciner, 2001; Ziegler *et al*., 2022; Aziz *et al*., 2023).

3-9-2 Correlation between TG/HDL Ratio and immunological Biomarkers for IHD Patients.

Correlation analysis between TG/HDL Ratio and immunological biomarkers for IHD Patients are illustrated in Table (3-7). The results of statistical analysis demonstrated a trend toward significant positive correlation between TG/HDL_Ratio and sIL-6R (p= 0.059). Also sIL-6R showed significant positive correlation with IL-6.

Table (3-7): Correlation between TG/HDL Ratio and Immunological Biomarkers for IHD Patients.

Parameters	Value	TG/HDL_Ratio	IL-6	sIL-6R	TNF-α
TG/HDL_Ratio	R.	1.000	-.169	-.245	-.014
	P.		.196	.059	.914
IL-6	R.		1.000	.400**	.019
	P.			.002	.884
sIL-6R	R.			1.000	.106
	P.				.422

*. Correlation is significant at the 0.05 level.
**. Correlation is significant at the 0.01 level.
Spearman's correlations were performed to assess the association strength and direction between the two continuous variables.

Increased serum TG and decreased HDL levels have been associated with metabolic syndrome and CVD, and their ratio, TG/HDL, has been proposed as a novel biomarker for predicting the risk of both clinical entities (Kosmas *et al*., 2023). Patients in the maximum quartile of TG/HDL ratio had the uppermost rate

of opposing cardiovascular proceedings, endothelial dysfunction and atherosclerosis, oxidative stress, pro-inflammatory status, and proliferation of vascular smooth muscle cells (Kundi *et al*., 2017). In cross-sectional study involving over 5000 participants, anthropometric measures and blood pressure were taken and the patients were categorized according to their lipid ratios (TC/HDL ratio, LDL/HDL ratio and TG/HDL ratio). After adjusting for several variables (age, Sex, BMI and medical history), researchers assumed that the TG/HDL was the optimal indicator for identifying metabolic syndrome as compared to other ratios (Rezapour *et al*., 2018). Another study in revealed that the high TG/HDL ratio was related with a 2.12 times increased risk of developing metabolic syndrome (Abbasian *et al*., 2017). In another study in a Chinese population, it was shown that the TG/HDL ratio was also a virtue predictor for the incidence of coronary artery calcification (Wang *et al*., 2022). The TG/HDL-C ratio also seems to possess a role in the setting of non-obstructing coronary artery disease.

A previous study showed that the odds of having a history of heart attack in presence of elevated TG and TNF-α and IL-6 are approximately 8-fold higher as compared to heart attack with upgrading only in the levels of TNF-α and IL-6 (Tahir *et al*., 2021).

In particular, the interplay of pro-inflammation with lipid abnormalities in the risk of IHD, remains poorly understood; so further investigations are required to address this gap. To our knowledge, this is the first study that addressed the role of the correlation between TG/HDL and IL-6, sIL-6R, and TNF-α in patient with ischemic heart disease.

3-10 Receiver Operative Characteristic Curve Analysis

The Receiver Operative Characteristic Curve (ROC) analysis yielded a cut off value of IL-6, sIL-6R, and TNF-α for prediction of disease activity by biomarkers for IHD disease. The overall AUC, sensitivity, and specificity for IL-6, sIL-6R, and TNF-α were as follows: (0.738, 0.900, and 600), (0.994, 0.967, and 0.967), and (0.999, 0.983, and 0.697), respectively, as displayed in Table (3-8).

Table (3-8): Receiver Operative Characteristic Curve (ROC) based analysis

Parameters	Sensitivity	Specificity	Cut off Point	AUC
TC (mg/dL)	0.133	0.867	224.950	0.249
TG (mg/dL)	0.200	0.933	201.050	0.376
HDL (mg/dL)	0.017	0.900	70.000	0.270
LDL (mg/dL)	0.300	0.833	40.200	0.458
IL-6	0.900	0.600	1.562	0.783
sIL-6R	0.967	0.967	16.954	0.994
TNF-α	0.983	0.697	108.485	0.999

The present study was designed to compare discrimination for IHD risk by different cut-off values of TC, TG, LDL, HDL, IL-6, sIL-6R, and TNF-α. To investigate whether studied variables could provide better prediction of IHD, we performed receiver operating characteristics analyses. The current results indicates that circulating IL-6, sIL-6R, and TNF levels are more accurate to predict IHD than concentrations of lipid profile, all were assessed in IHD, demonstrated by a higher Area Under the Curve (AUC) for IL-6, sIL-6R, and TNF (= 0.783, 0.994, 0.999 respectively, vs AUC for TC, TG, HDL, and LDL (= 0.249, 0.376, 0.270, 0.458 respectively). The AUC is a numerical measure that can vary from 0 to 1. However, it is important to note that only values greater than 0.5 are deemed to have diagnostic significance. A higher value enhances the overall effectiveness of the test (Nahm, 2022); the minimal AUC, which is equal to 0.5, should be considered as a measure of chance; so and as a result of these findings we speculated that IL-6, sIL-6R, and TNF levels are more accurate to predict IHD than concentrations of lipid profile. The trend is similar with what has been found in a previous study about IL-6 in order to predict CAD (AUC = 0.74) (Wainstein *et al*., 2017; Gager *et al*., 2020).

In figure (3-9) we calculated the optimal cut-off point of IL-6 (1.562), depending on the greatest sum of sensitivity and specificity of the ROC-coordinate points; which is in contrast to what has been reported in previous literature (Gager *et al*., 2020). Current study found that the area under the curve for IL-6 were 0.783, which higher than that obtained in study of Zegeye *et al* (2021). The present findings agrees with a previous study that considered IL-6 as a reasonably accurate indicator for predicting the likelihood of IHD in patients, with a sensitivity of 90.0% and specificity of 60.0% at the chosen cut-off, so this statistically significant marker can be used to diagnose positive cases of the condition (Obuchowski and Bullen, 2018) (Mossmann,et al., 2022) .

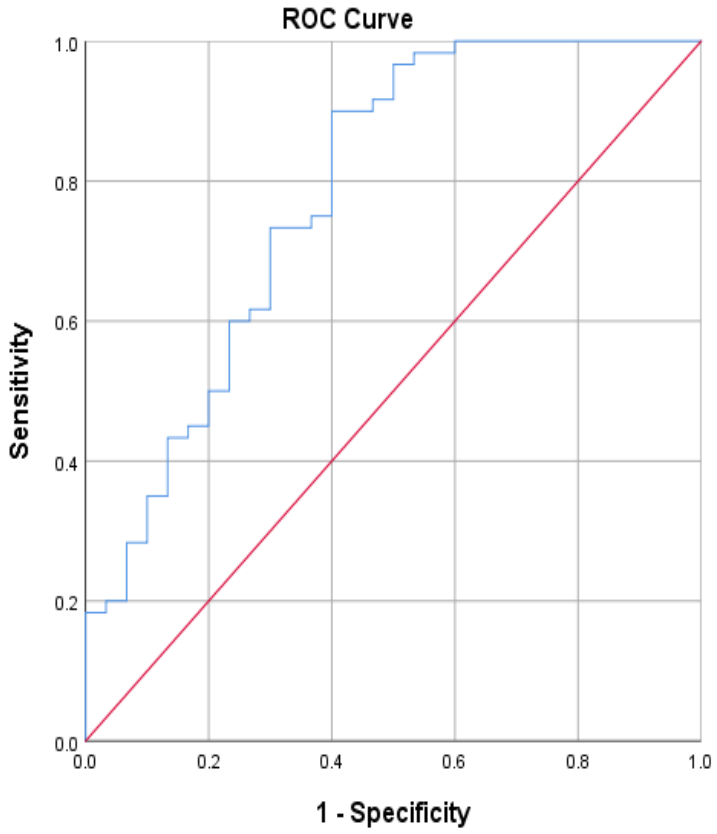


Figure (3-9): Receiver operating characteristic Curve (ROC) illustrating the sensitivity and 1-specificity values for IL-6.

In figure (3-10) the current investigation consistent with study of (Polo and Miot, 2020), who showed that sensitivity and specificity of sIL-6R were both 96.7% at the chosen cut-off point, indicating that it is an excellent predictor for the expectation of ischemic heart disease. This means that sIL-6R is a reliable measure for distinguishing a positive test result. Even so, comparable results were documented in previous studies that showed that the AUC, sensitivity, and specificity of IL-6 was (100%, 100%, and 1.00), (Polo and Miot, 2020).Another previous study showed that the sensitivity and specificity for IL-6 was was (100%, 100%, and 1.00), (Hajian -Tilaki, K. 2013).

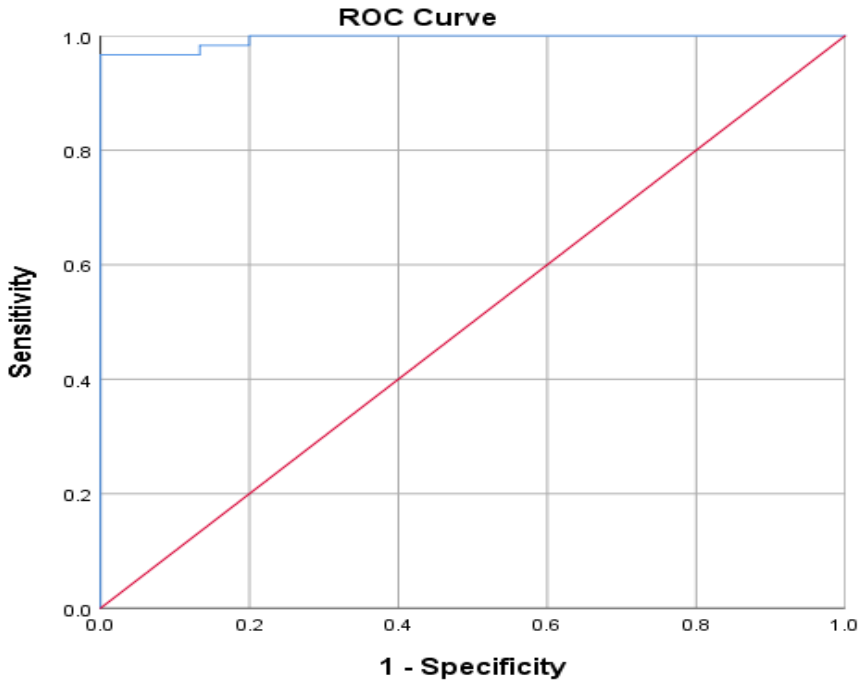


Figure (3-10): Receiver operating characteristic Curve (ROC) illustrating the sensitivity and 1-specificity values for sIL-6R.

In addition, TNF-α is considered a strong predictor for the expectation of ischemic heart disease in patients, with a sensitivity of 98.3% and specificity of 69.7% at the chosen threshold. Statistically, this indicates that the marker is relevant in diagnosing a positive case as shown in Figure (3-11).

On the contrary, sIL-6R and TNF-α exhibit promising diagnostic capabilities for ischemic heart disease, with outstanding AUC values as well as high sensitivity and specificity, additionally, a previous study revealed that the sensitivity and specificity of TNF-α was (100%, 100%, and 1.00), (Obuchowski and Bullen, 2018).

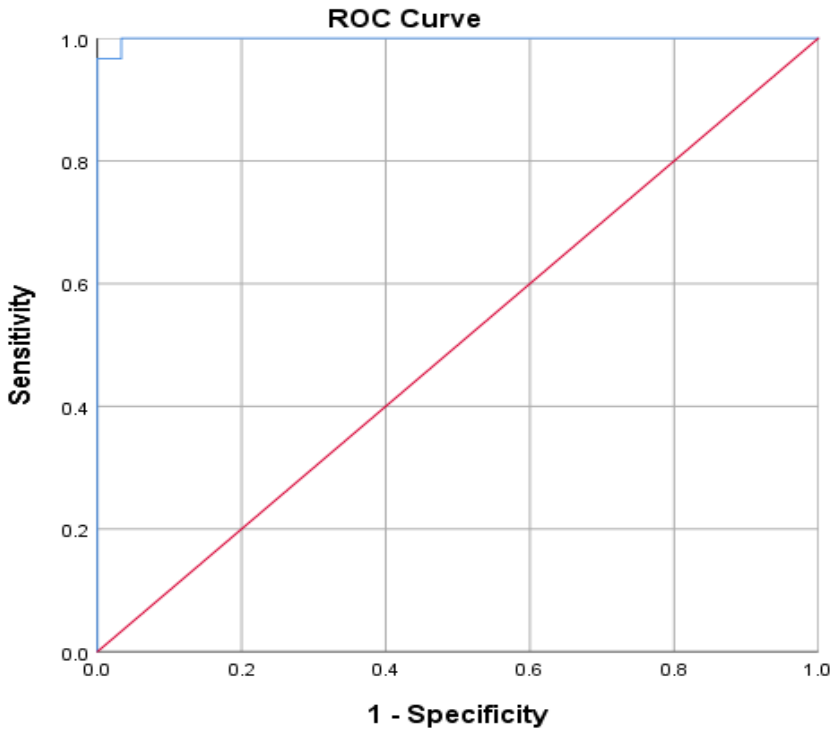


Figure (3-11): Receiver operating characteristic Curve (ROC) illustrating the sensitivity and 1-specificity values for TNF-α.

The differences in sensitivity and specificity values could be due to patients and control subjects chosen, differences according to population ethnicity, and the differences in kits and techniques used in these studies.

Conclusions and Recommendations

conclusions

1. The current study showed a relationship between immunological markers and lipid profile and the onset of IHD.
2. The current results indicate that circulating IL-6, sIL-6R, and TNF levels are more accurate to predict IHD than lipid profile.
3. Aging can be a trigger factor for the emergence of IHD.

Recommendations

1. Evaluate another biochemical parameter such as oxidative stress parameters and assess their relation with IHD development.
2. Investigate the potential interplay between the studied biomarkers and the genetic variation in IHD patients.
3. Further investigations and continuous improvement of detection technologies about anti IL-6-related drugs are needed to lessen its role in IHD development.
4. The current study recommended with using of numerous effectual interventional strategies as well primary preventive practices against IHD and effective disease screening to improve the quality of life among patients with IHD.
5. Continuous pursuit of the exercise and weight loss.
6. The study recommends the importance of including inquiries about patients' physical activity conditions and the extent of their relationship to changing lipid parameters in IHD patients.
7. IL-6 and TNF-α control strategy may provide a new therapeutic prospective for IHD.

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Appendixes

No.	Name	Sex	Age	Weight	Length	History Of family	Blood pressure	**Notes**

الخلاصة

الخلاصة

مرض الشريان التاجي (IHD) هو حالة تنجم عن الانسداد الشديد لنقل الدم إلى عضلات القلب. يعد ملف الدهون والاضطرابات المناعية من أهم عوامل الخطر للإصابة بـ IHD. أجريت الدراسة الحالية خلال الفترة من ديسمبر 2022 إلى مايو 2023؛ وشملت جمع عينات الدم من (30) فردًا تم اختيارهم عشوائيًا كمجموعة ضوابط تتراوح أعمارهم بين 40-85 عامًا وكذلك (60) مريضًا يعانون من IHD تتراوح أعمارهم بين 40-85 عامًا، والذين كانوا يزورون مستشفى الإمام الحسين الطبي المدينة وكذلك مستشفى الإمام الحسن المجتبى في محافظة كربلاء. تم جمع عينات الدم من جميع الأفراد لتقييم ملف الدهون الذي شمل (الكوليسترول الكلي، الدهون الثلاثية، البروتين الدهني عالي الكثافة، البروتين الدهني منخفض الكثافة، نسبة الدهون الثلاثية إلى البروتين الدهني عالي الكثافة) والبيومركرات المناعية (بما في ذلك الإنترلوكين -6، مستقبل الإنترلوكين -6 القابل للذوبان، عامل نخر الورم -الفا).

أظهرت الدراسة فروقًا كبيرة بين حالات IHD والضوابط الصحية. كانت حالات IHD ذات نطاق عمري أكبر، وكتلة مؤشر كتلة الجسم أعلى، ومستويات ضغط الدم مرتفعة مقارنة بالضوابط. أظهر التحليل البيوكيميائي زيادة في الكوليسترول الكلي و الدهون الثلاثية و البروتين الدهني منخفض الكثافة ، مع انخفاض البروتين الدهني عالي الكثافة ، في مرضى IHD. كما ارتفعت المعلمات المناعية بشكل كبير ($P\leq0,05$) لدى مرضى IHD.

درست الدراسة توزيع السكان المدروسين حسب العمر، ووجدت أعلى نسبة من مرضى IHD والضوابط في الفئة العمرية ≤60 وأدنى نسبة في الفئة العمرية ≥50. كما درست تأثير العمر على ملف الدهون والبيومركرات المناعية في IHD. أظهرت النتائج أن مستويات (الدهون الثلاثية) و (البروتين الدهني منخفض الكثافة) ارتفعت بشكل كبير ($P\leq0.05$) مع تقدم العمر، حيث كانت أعلى في الفئات العمرية الأكبر. ومع ذلك، لم تختلف بقية الدهون والبيومركرات المناعية بشكل كبير عبر الفئات العمرية.

وجدت الدراسة أن الجنس له تأثير كبير على مستويات البروتين الدهني منخفض الكثافة في مرضى IHD، حيث أظهرت الإناث مستويات البروتين الدهني منخفض الكثافة أعلى مقارنة بالذكور. ومع ذلك، لم يكن للجنس تأثير كبير على بقية المؤشرات البايوكيميائية للدهون أو على المؤشرات المناعية في كل من مرضى IHD والضوابط الصحية.

وجدت الدراسة ارتباطات كبيرة بين العديد من المؤشرات الحيوية. ارتبط الطول بشكل إيجابي بالوزن، بينما ارتبط الدهون الثلاثية بشكل إيجابي بـ الكوليسترول الكلي . أظهر البروتين الدهني عالي الكثافة ارتباطًا عكسيًا مع الطول و الكوليسترول الكلي . ارتبط البروتين الدهني منخفض الكثافة بشكل إيجابي بـ الكوليسترول الكلي و الدهون الثلاثية. ارتبط sIL-6R بشكل إيجابي بـ IL-6. أظهرت نسبة TG / HDL اتجاهًا ارتباطًا إيجابيًا مع sIL-6R. كما حددت الدراسة قيمًا قصوى IL-6 و sIL-6R و TNF-α للتنبؤ بنشاط المرض.

بشكل عام، تسلط النتائج الضوء على أهمية المؤشرات البيوكيميائية للدهون، خاصة نسبة TG / HDL ، وكذلك IL-6 و TNF-α في تطور IHD في المجتمع العراقي. تعد مستويات IL-6 و sIL-6R و TNF-α المتداولة مؤشرات أكثر دقة لـ IHD من تركيزات ملف الدهون.

جامعة كربلاء

كلية العلوم

قسم علوم الحياة

تقييم مستوى الدهون وبعض المعايير المناعية في مرضى نقص التروية القلبية في محافظة كربلاء

رسالة مقدمة

الى مجلس كلية العلوم / جامعة كربلاء

وهي جزء من متطلبات نيل درجة الماجستير في علوم الحياة

من قبل

زينب محمد نعمة الحسيني

بكالوريوس علوم الحياة – جامعة كربلاء - 2005

بإشراف

الأستاذ المساعد الدكتور

عفاف خيري اسماعيل ابراهيم

ايار 2024م ذو القعدة 1445ه