T.C.
KIRSEHIR AHI EVRAN UNIVERSITY
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF CHEMISTRY

EXPLORATION the RELATIONSHIP BETWEEN SALUSIN-α, SALUSIN-β and IRISIN LEVELS in PATIENTS with HEART DISEASE in AL-ANBAR STATE

AYMEN FARIS HAMMOOD

MASTER THESIS

KIRŞEHİR / 2022

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ADVISOR
Prof. Dr. Aslıhan GÜNEL

CO-ADVISOR
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THESIS STATEMENT

I certify that all information in the thesis was obtained and presented within the framework of ethical conduct and academic rules, and in this study prepared according to the rules of dissertation writing, all types of statements that do not belong to me are fully cited as the source of the information.

Aymen Faris Hammood

Based on Articles 9/2 and 22/2 of the Graduate Education and Training Regulations published in the Official Gazette on 20/4/2016; A report was obtained according to the criteria set by the Institute of Science and Technology using the Plagiarism Program program which Kırşehir Ahi Evran University has subscribed to this postgraduate thesis.

PREFACE

I would like to thank my esteemed advisor Prof. Dr. Aslıhan GÜNEL, from whom I learned how a scientist should work, as well as being an example to me with her calm and patient manner that she has shown since the day I met her during my master's. I would like to thank my esteemed co-advisor Prof. Dr. Khalid FAROUQ. In addition, I would like to thank Assoc. Prof. Dr. Zuhal Alım and Prof. Dr. Nadir Demirel for their kind help and supports.

I dedicate my dissertation to my family.

October, 2022 **Aymen Faris Hammood**

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LIST OF ABBREVIATIONS

Symbols	Description
%	: Percent
°C	: Cantigrade
L	: Litre
pg	: Picogram
dl	**:** Desiliter
Kg	: Kilogram
mg	**:** Miligram
mL	: Mililiter
µg	: Microgram

Abbreviations	Description
ACC	**:** After cardiac catheterization
ACTH	**:** Adrenocorticotropic hormone
ADA	**:** American Diabetes Association
ADP	**:** Adenosine 2 Phosphates
AMI	**:** Acute Myocardial Infarction
APO	**:** Apolipoproteins
BCC	**:** Before Cardiac Catheterization
BMI	**:** Body Mass Index
CAD	**:** Coronary Artery disease
CCU	**:** Coronary Care Unit
CHD	**:** Congenital Heart Disease
CT	**:** Computed tomography
CVI	**:** Cerebral Vascular Injury
CVD	**:** Cardiovascular disease
Da	**:** Dalton
DM	**:** Diabetes Mellitus
ELISA	**:** Enzyme Linked Immuno Sorbent Assay
FNDC5	**:** Fibronectin type 3 domain-containing protein 5
Fnlll	**:** Fibronectin type III
G-3-P	**:** Glycerol 3 phosphate
GAD	**:** Glutamic acid decarboxylase
GDM	**:** Gestational Diabetes Mellitus
GK	**:** Glycerol Kinase
GOD	**:** Glucose Oxidase
GPO	**:** Glycerol -3- Phosphate Oxidase
HbA1c	**:** Hemoglobin A1c
HD	**:** Heart Disease
HDL	**:** High Density Lipoproteins
HF	**:** Heart Failure
HLA	**:** Human Leukocyte Antigen
HMGCR	**:** Hydroxy-3-Methyl-Glutaryl Coenzyme A Reductase
HOMA-IR	**:** Homeostasis model assessment insulin resistance
HPAA	**:** Hypothalamic-pituitary-adrenal axis
HT	**:** Hypertention
IFG	**:** Impaired fasting glucose

IGT : Impaired glucose tolerance
IHD : Ischemic heart disease
IR : Insulin Resistance
LDL : Low Density Lipoproteins
LDLR : LDL Receptor
LPL : Lipoprtein Lipase
MRDM : Malnutrition Related Diabetes Mellitus
mRNA : Messenger Ribonucleic Acid
MW : Molecular Weight
NIDDM : Non-Insulin Dependent Diabetes Mellitus.
OGTT : Oral Glucose Tolerance Test
PAD : Peripheral Artery Disease
PC2 : Prohormone Convertase 2
PC3 : Prohormone Convertase 3
PCA : Posterior Cerebral Artery
POD : Peroxidase
r : Correlation coefficient
RER : Rough endoplasmic reticulum
RIA : Radioimmuno assay
SD : Standard deviation
SUA : Serum uric acid
T1DM : Type 1 Diabetes
T2DM : Type 2 Diabetes
TC : Triglyceride
TG : Total cholesterol
TOAST : Trial of ORG 10172 in Acute Stroke Therapy
US : United States
VLDL : Very low density lipoproteins
WHO : World Health Organization

ÖZET

YÜKSEK LİSANS TEZİ

AL-ANBAR İLİNDE KALP HASTALIĞI OLAN HASTALARDA SALUSİN-α, SALUSİN-β ve İRİSİN DÜZEYLERİ ARASINDAKİ İLİŞKİNİN ARAŞTIRILMASI

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Kalp hastalığı dünya çapında başlıca ölüm nedenleri arasındadır. Kardiyovasküler hastalık (KVH), miyokard kaslarını ve kalbi besleyen dolaşım sistemini etkileyenler de dahil olmak üzere çeşitli sorunları içermektedir. KVH, ateroskleroz ile bağlantılı olmakla beraber orta arterlerle ilişkili olarak lipid ve fibröz elementlerin birikmesi ile karakterize inflamatuar bir hastalıktır. Bu tez çalışmasında 35 yaş ve üzeri 85 Iraklı gönüllü için bir vaka kontrol çalışması tasarlanmıştır. Bunlardan 25'i genellikle KVH öyküsü olmayan "sağlıklı" bireylerken, 60'ı kalp hastasıydı. Bu hastalar Irak Anbar Hastanelerindeki "Kalp Bakım Ünitesi"nde kardiyovasküler hastaları olarak kayıtlıdır. Mevcut çalışmanın amacı, salusin-α, salusin-β, irisin, lipid profili, açlık serum glukozu, insülin hormonuna dayalı insülin direncinin (IR) yanı sıra, kalp kateterizasyonundan (BCC) öncesi ve kalp kateterizasyonundan (ACC) sonrasında uygulanan KVH hastalarının serumlarında bir homeostatik modelin (HOMA-IR) değerlendirilmesi ve bu düzeyleri kendi aralarında ve ayrıca sağlıklı (kontrol) grubundakilerle karşılaştırmaktır. Parametrenin yaş ve VKİ alt grubuyla karşılaştırılmasına ek olarak, mevcut veriler sağlıklı kontrol grubu ile karşılaştırıldığında ACC ve BCC gruplarında Salusin-β'da önemli ölçüde daha yüksek bir seviye ve salusin-α ve irisin'de anlamlı olarak daha düşük seviyeler görüldü. ACC grubu

ile karşılaştırıldığında BCC grubunda da aynı sonuçlar bulunmuştur. Sonuçlarımızın istatistiksel analizi, salusin-α, salusin-β ve irisin'in sırasıyla 95 pg/ml, 214 pg/mL ve 1.3 µg/mL eşik değerleriyle ve sırasıyla %97.8, %100 ve %85.7 duyarlılık ve %97.4, %95.2 ve %97.4 özgüllük ile klinik uygulamalarda Irak popülasyonunda KVH riskleri için bir tanı aracı olarak kullanılabileceğini göstermiştir. ACC ve BCC gruplarında elde edilen sonuçlar, sağlıklı kontrol grubu ile karşılaştırıldığında glukoz, insülin, HOMA-IR, TC, LDL, TG, VLDL seviyelerinde de anlamlı bir artış olduğunu göstermiştir. BCC hastaları ile ACC hasta grupları karşılaştırıldığında, TC, LDL, TG ve VLDL'de önemli ölçüde daha yüksek bir seviye ile birlikte HDL seviyesinde bir düşüklük söz konusu olmakla beraber glukoz, insülin ve HOMA-IR'de anlamlı olmamıştır. Ayrıca bulgularımız, aşırı kilo ve obezitenin, özellikle yetişkin erkek obez bireylerde KVH için en önemli risk faktörü gibi göründüğünü göstermekte olup bu çalışma, Irak popülasyonuna dayalı araştırmaların salusin-α, salusin-β, irisin ve HOMA-IR'nin genel popülasyonda aterosklerozu saptamak için iyi bir aday tanı belirteci olabileceğini göstermektedir.

Ekim 2022, 69 Sayfa

Anahtar Kelimeler: Salusin-α, Salusin-β, İrisin, İnsülin, Kalp Hastalığı, HOMA-IR

ABSTRACT

M.Sc. THESIS

EXPLORATION the RELATIONSHIP BETWEEN SALUSIN-α, SALUSIN-β and IRISIN LEVELS in PATIENTS with HEART DISEASE in AL-ANBAR STATE

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The primary causes of death worldwide are heart disease (HD). Cardiovascular disease (CVD) includes a variety of problems, including those affecting the myocardial muscles and the circulatory system that supplies the heart. CVD is as a rule connected with atherosclerosis; an inflammatory disease characterized via the accumulation of lipids and fibrous elements in relation to medium arteries. In this thesis study, a case-control study was designed for 85 Iraqi volunteers aged 35 and over. Of these, 25 were generally "healthy" with no history of CVD, while 60 Iraqi patients with CVD. These patients are registered as CVD patients in the "Cardiac Care Unit" in Anbar Hospitals, Iraq. The aims of the present is to measure the levels of salusin-α, salusin-β, irisin, lipid profile, as well as assessment of insulin resistance (IR) based on fasting serum glucose, insulin hormone, and a homeostatic model (HOMA-IR) in the serums of CVD patients who are subject to before cardiac catheterization (BCC) and after cardiac catheterization (ACC) and to compare these levels between them and also with the ones of the healthy (control) group. In addition to compare each parameter with subgroup of age and BMI. The present data showed a significantly higher level in the salusin-β and significantly lower levels in the salusin-α and irisin with ACC and BCC groups when compared with that of the healthy control group.

The same results were also found in BCC group when compared with ACC group. The statistical analysis of our results showed the salusin-α, salusin-β and irisin, can be used as a diagnostic tool for CVD risks in Iraqi population in clinical practice with the cut-off values of 95 pg/mL, 214 pg/mL and 1.3 µg/mL respectively, with sensitivities of 97.8%, 100% and 85.7% and specificities of 97.4%, 95.2 and 97.4%, respectively. The results in patients with ACC and BCC groups have also shown that there is a significant increase in the glucose, insulin, HOMA-IR, TC, LDL, TG, VLDL levels when compared with that of the healthy control group. When comparing BCC patients with ACC patients groups there were a significantly higher level in the TC, LDL, TG, and VLDL accompanied with a low in the HDL level and non-significant in the glucose, insulin and HOMA-IR. Moreover, our findings indicate that overweight and obesity appears to be the most important risk factor for CVD, especially in adult men obese individuals, and this study suggests Iraqi population-based research have shown that salusin-α, salusin-β, irisin and HOMA-IR might be a good candidate diagnostic marker for detecting atherosclerosis in the general population.

October 2022, 69 Pages

Keywords: Salusin-α, Salusin-β, Irisin, Insulin, Heart Disease, HOMA-IR

1. INTRODUCTION

1.1. Cardiovascular Disease

Cardiovascular disease (CVD) may result in serious issues with global health. Congenital cardiac disease and cerebral vascular disease are also included in CVD. Atherosclerosis, the most prevalent real cause of CVD, accounts for the majority of the disease (Zhong, S. *et al.*, 2019). The bulk of non-infectious illness deaths, which total 17.7 million annually worldwide, are caused by cardiovascular disorders, particularly in low- and middle-income nations (Saeed, K. M. I. *et al.*, 2020). CVD ranks first as a cause of disease-related death in Iraq. Nowadays, in developing countries, including Iraq, there are alarming signals of a higher rate of CVD at young age (Mohammad, A. M. *et al.*, 2021).

Among the most important CVD risk factors are lipid abnormalities. Total cholesterol (TC), low-density lipoprotein (LDL), high density lipoprotein (HDL), and triglyceride (TG) diseases are all referred to as metabolic disorders (hyperlipidemia). Adiposity is also linked to a higher risk of CVD than people with a healthy BMI (Zhong, S. *et al.*, 2019). Obesity has been implicated in the development of insulin sensitivity. Insulin resistance (IR) is defined as just an impaired physiologic response to insulin, as evidenced by deficits in glucose absorption and oxidation, a reduction in glycogen synthesis, as well as the capacity to control the oxidation process (Ormazabal, V. *et al.*, 2018). Even though the majority of the populations who are resistant to insulin also are obese or overweight, this is not the case in the general population. CVD, type 2 diabetes mellitus (T2DM) and hypertention (HT) are all much more likely in only one of third of the population who are IR. Obese people are much more likely to have HD due to a decrease of insulin sensitivity, although not everyone who is obese or overweight is IR (Reaven 2011; Matthews, D. R. *et al.*, 1985).

On the other hand the progression of atherosclerosis can also be exacerbated by the usual cardiac health problems such as diabetes mellitus (DM), hypertensive (HT), overweight, hyperlipidemia and hereditary factors, among affect things (Raggi, P. *et al.*, 2018). Obesity

has been more common around the world in recent decades. Obesity causing atherosclerotic, inflammatory responsive and cardiac damage (Bastien, M. *et al.*, 2014).

Several diagnostic biomarkers such as troponin I and T, c-reactive protein and D-dimer for CVD are known (Ghantous, C. M. *et al.,* 2020), these are late stage biomarkers and also myeloperoxidase, Serum amyloid A and vitamin D-binding protein are known as some early stage biomarkers (Ghantous, C. M. *et al.*, 2020). Salusins-α and/or salusin-β may also important associated atherosclerosis (Sato, K. *et al.*, 2013). In addition irisin, a recently found like myokine, has indeed been found to be an early diagnosis marker in a variety of CVD cases (Silvestrini, A. *et al.*, 2019). The function of salusin-α, salusin-β levels, and irisin in heart failure patients (HF) undergoing catheterization in Iraq is little understood or unknown. Therefore, the purpose of this thesis is to offer fresh information on the correlation between the levels of lipid profiles and salusin-α, salusin-β, irisin, and IR in HD patients in the governorate of Al-Anbar, Iraq. Early cardiac catheterization diagnosis is crucial, thus the research of salusin-α, salusin-β, and irisin may be a promising potential diagnostic marker for identifying atherosclerosis as a predictive tool in patients with CVD undergoing cardiac catheterization in clinical practice in the Iraqi population.

1.2. Objectives

1. To investigate whether serum salusin-α and salusin-β levels are associated with HD.
2. To investigate the irisin, is a marker of atherosclerosis in patients with HD.
3. To recognize if these variables can be used to predict or reduce the incidence of HD.
4. To determine the usefulness of the HOMA-IR in measuring IR in individuals with HD and if there were any correlation with CVD.
5. To investigate the relationship between BMI as well as IR in individuals with HD compared with healthy.
6. To evaluate the lipid panel (TC, TG, HDL, LDL, and VLDL) within serum of HD patients to that of healthy people, to see if there was a link involving serum biochemical markers hormonal and the blood lipids.
7. To evaluate the strength of the connection between fasting blood glucose, insulin, andHOMA-IR with clinical HD.

2. GENERAL PARTS

2.1. Cardiovascular Diseases

2.1.1. Definition

The term CVD refers to any condition that affects blood circulation, which includes the heart and the arteries. Multiple congenital and acquired conditions are involved in this complex disorder. Atherosclerosis, cardiomyopathy, heart failure (HF) as well as angina pectoris, and congenital cardiovascular malformations are examples of CVD. Primary health care facilities frequently receive patients with these problems (Touray, M., and Touray, A., 2021).

Hyperlipidemia, smoking, a sedentary lifestyle, a poor diet, HT and obesity can all raise the risk of CVD (Rychter, A. M. *et al.*, 2020).

2.1.2. Morbidity and Mortality

The number of people who have died as a result of CVD is growing, and there are many people in the globe who have the condition. Up to 4 million people worldwide each year succumb to this illness. Numerous studies have demonstrated a significant correlation between gender and the severity or prevalence of the condition. Males are more prone than females to contract a sickness (Rychter *et al.*, 2020).

The bulk of deaths from non-contagious illnesses are caused by CVD. Every year, 17.7 million people around the world are impacted by it, mostly in developing countries. The leading factor in cardiac fatalities worldwide is CAD, which is responsible for over 4.5 million deaths in the world's population (Mohammad, A. M. *et al.*, 2021).

2.1.3. Types of Cardiovascular Disease

CVD can be present from birth or develop during the duration of a person's life. In terms of CVD, it includes atherosclerosis, which includes coronary heart disease(CHD), cerebral artery disease and peripheral artery disease, as well as two major complications, heart

attack and ischemic stroke (the latter of which is more common than hemorrhagic stroke) (Touray, M., and Touray, A., 2021; Mendis, S. *et al.*, 2011).

2.1.3.1. Coronary Artery Disease

The hallmark of a condition brought on by CHD or ischemic heart disease (IHD) is a drop in arterial blood flow, which can cause symptoms such hidden ischemia, angina, or cardiogenic shock (sudden cardiac death) (Mishra, N. K. *et al.*, 2016). CHD continues to be the leading cause of death in all socioeconomically varying countries. Over 9 million people died in 2016, according to estimates from the World Health Organization (Consultation, W. H. O. 2000), as a result of discrepancies in death rates (Nowbar, A. N. *et al.*, 2019). According to epidemiological reports from 2016, Iraq was expected to have a 33 percent increase in CVD deaths, while Saudi Arabia and Kuwait had the highest incidence rates of IHD (46 and 41 percent, respectively) (Kalaf, H. *et al.*, 2016; Mokdad, A. *et al.*, 2018).

CAD is brought on by plaque build-up in the blood arteries that return blood to the heart. Plaque is created by lipid encrustations. The arteries narrow with time as a result of plaque formation. The medical word for this ailment is atherosclerotic. The illness atherosclerosis is brought on by intricate lesions in the cerebral, peripheral, and cardiac arteries. The pathogenesis of atherosclerosis includes endothelial dysfunction, cholesterol accumulation, inflammation, smooth muscle cell hyperplasia, and calcification. For a very long time, it has been believed that lipids are the primary factor in the formation of atherosclerosis (Wang, T., and Butany, J., 2017; Wang, H. H. *et al.*, 2017).

When fat-rich foam cells gather in the artery's endothelium and create a "fatty stripe," the condition is known as an atherosclerotic lesion. The main arteries' wall first develops dangerously high levels of lipid retention, followed by persistent inflammation at those locations, which causes fat streaks and ultimately causes fibrous hardening of the arteries. The first step in the pathogenesis of atherosclerosis is lipid preservation (figure 2.1). Three components—inflammation cells, smooth muscle cells, fibrous components of connective tissue, and a fat element made of lipids—make up atherosclerotic lesions, which are created from fatty streaks (Brown, R.A. *et al.*, 2017; Wang, T., and Butany, J., 2017; Aziz, M., and Yadav, K. S., 2016).

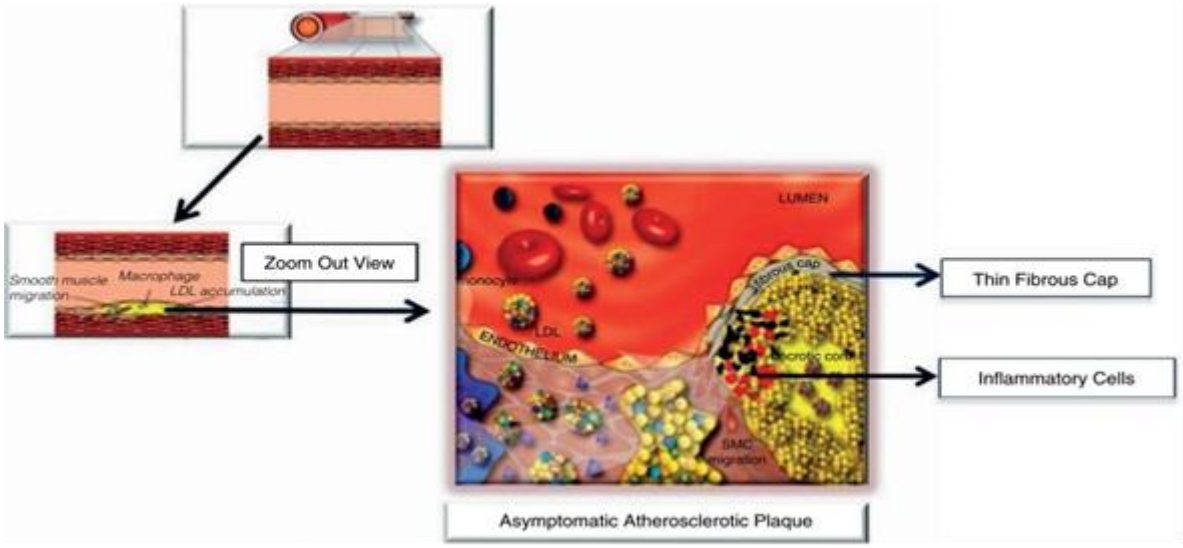


Figure 2.1. Diagram shows an artery and the formation of an asymptomatic atherosclerotic plaque (Ambrose, J. A., and Singh, M., 2015).

Plaques produce constriction inside the blood vessels that results in the fatal occurrences of an acute coronary syndrome, infarction, fatal arrhythmia, and sudden cardiac death (figure 2.2) (Aziz, M., and Yadav, K. S., 2016; Wang, T., and Butany, J., 2017).

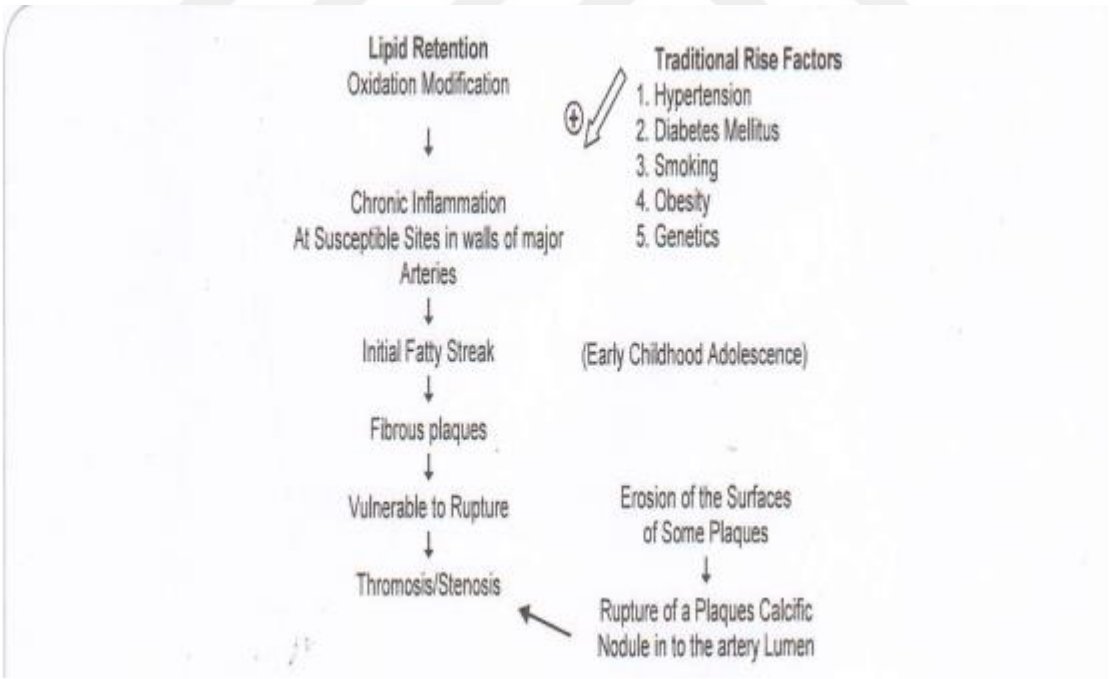


Figure 2.2. Stages of atherosclerosis (Aziz, M., and Yadav, K. S., 2016).

Lifestyle risk factors, such as being overweight, can speed up the atherosclerosis processes (Figure 2.3). To ascertain whether a patient has ischemia, a patient's history, clinical evaluation, fasting lipid panel, fasting blood glucose, and hemoglobin A1c (HbAlc) should

all be carried out. Patients who have received a diagnosis of sickness at one facility should be examined elsewhere. Digital Tomography A common first-line screening diagnostic for CAD is angiogram. Other diagnostic techniques are used in addition to needle diagnostics, including intravascular ultrasonography, angioscopy, plaque thermography, sonography, and immunoscintigraphy (Aziz, M., and Yadav, K. S., 2016).

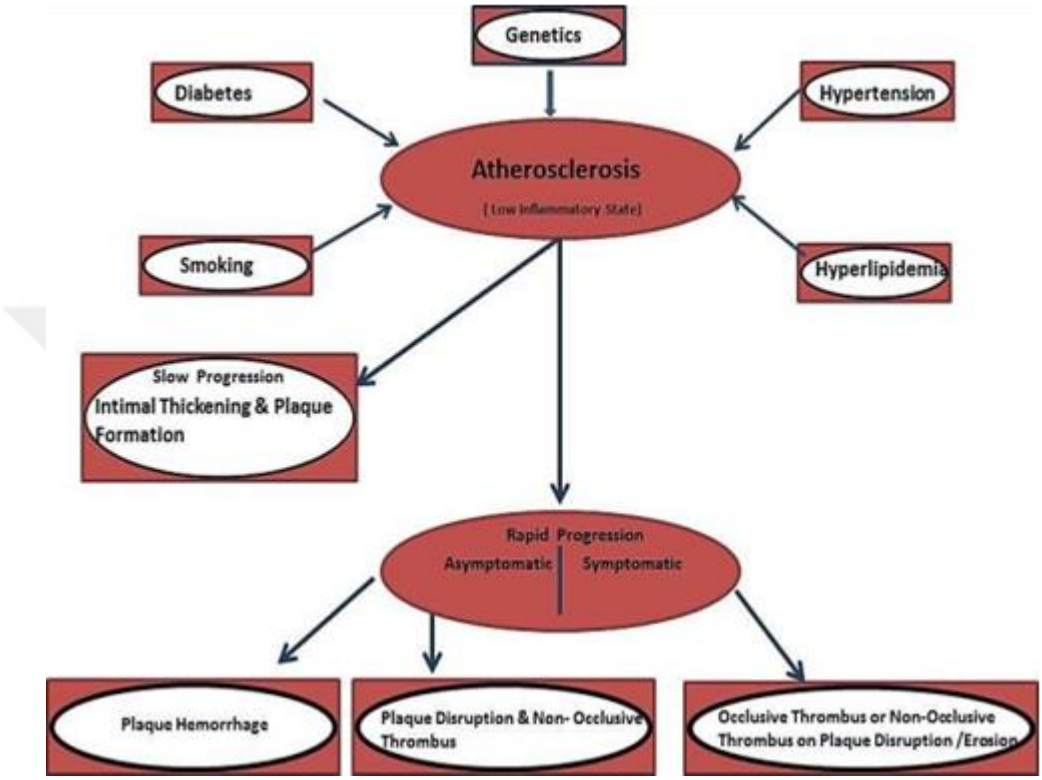


Figure 2.3. Diagram showing risk factors and progression of atherosclerosis (Ambrose, J. A., and Singh, M., 2015).

2.1.3.2. Cerebrovascular Injury

A stroke is referred to as a Cerebral Vascular Injury (CVI) in medical terms. Basically, it's a neurologic deficiency caused via a vascular source causing an immediate localized insult to the nervous system. Strokes are indeed the fifth main cause of death in the United States (US) when exclude other HD from the equation (Hui, C. *et al.*, 2021).

Approximately 6.3 million people died from strokes in 2015, making it the second most frequent condition to cause mortality after coronary artery disease (11 percent of the total). Hemorrhagic stroke caused about 3.3 million fatalities worldwide, while ischemic stroke caused about 3.0 million fatalities (Wang, H. *et al.*, 2016). The two main types of CVI are

ischemic strokes, which are brought on by a blockage in the brain, and hemorrhagic strokes, which are brought on by a burst blood vessel inside the brain.

2.1.3.2.1. Ischemic Stroke

Ischemic stroke has been the most frequent type of strokes, and it occurs when blood supply to the brain is interrupted by a block in the artery surrounding the brain. In the US, ischemic stroke is the sixth highest cause of mortality in the population (Hui, C. *et al.*, 2021). One of the most common causes of ischemic stroke is a clot in the bloodstream, which prevents enough blood from reaching the brain. Primary care tries to recanalize the blocked main artery as soon as feasible to restart blood flow. Anesthesia is used to accomplish this (Staessens, S., and De Meyer, S. F., 2021).

For the purpose of determining the incidence of an ischemic as well as the degree and location of the stroke, acute stroke therapy using the TOAST (Trial of ORG 10172 in Acute Stroke Therapy) guidelines is divided into four etiological subclasses: stroke due to major arterial atherosclerosis (TOAST 1), cardioe mbolic attack (TOAST 2), other identified cause (TOAST 4) and strokes of unknown etiology (TOAST 5). The final subtype (TOAST 5) encompasses all patients with an inadequate assessment or an etiology that has not been identified (Boodt, N. *et al.*, 2020).

2.1.3.2.2. Hemorrhagic Stroke

Hemorrhage is produced by bleeding from injured vessels, which occurs as a result of vascular permeability within ischemic myocardium or vascular burst as a result of ischemia. Intracerebral hemorrhage (ICH) is a type of stroke that occurs when blood from an artery source enters the brain parenchyma completely. It represents for 5-15 percent of all strokes. The leading reason is HT; however other factors such as age, ethnicity, smoking cigarettes, alcohol consumption, and increased serum TC have also been discovered. In certain cases, ICH develops in the absence of HT and in unusual sites. Small vascular abnormalities, vasculitis, brain cancer, and sympathomimetic medications are among the causes (e.g. cocaine). ICH can also be induced by cerebral amyloid angiopathy, and it is rarely triggered by abrupt problems with blood pressure, such as those produced by cold exposure. The increased use of anti- thrombotic treatment for ischemic illnesses of the brain, heart, and other organs influences the prevalence of ICH (Brainin, M., and Heiss, W. D., 2019).

No particular sign distinguishes ischemia from brain bleeding. Symptoms appear suddenly and thus are usually at their most severe within minutes. To distinguish ischemia from intracranial hemorrhage, all stroke patients require immediate brain imaging. To rule out cerebral bleeding or other reasons, non-contrast computed tomography (CT) figure (2.4) or magnetic resonance imaging, scanning, Doppler ultrasonography, and arteriography are required. The diagnosis of a stroke is done with the help of imaging tools. Stroke types and causes can also be determined using imaging techniques. Although blood test results may be useful in determining the likely aetiology of stroke, there is currently no widely utilized screening blood test for stroke assessment (Hill, M. D., 2005).

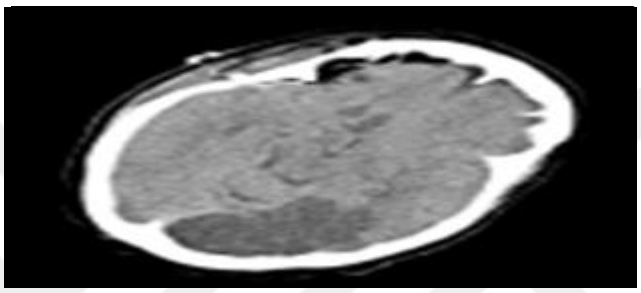


Figure 2.4. An axial section on noncontrast CT head shows left PCA ischemic stroke and no hemorrhage. PCA : Posterior Cerebral Artery (Hui, C. *et al.*, 2021).

2.1.3.3. Peripheral Artery Disease

Peripheral artery disease" refers to people who have atherosclerosis in all of their arteries but not in their coronary arteries (PAD). Twenty percent of adults over 60 have PAD, which is a rising epidemic. People with symptomatic PAD are more likely to get cardiovascular disease and die young since atherosclerosis is a disease that simultaneously affects several different body systems. One in eight people who had atherosclerotic disease had a severe CVD, and one in six people died in the first year after diagnosis (Sigvant, B. *et al.*, 2017).

Additional tests that may detect co-morbid diseases, such as DM, that require their own treatment are described in table 2.1 (Morley, R. L. *et al.*, 2018).

Table 2.1. History, examinations, and investigations for suspected PAD (Morley, R. L. *et al.*, 2018).

History •Previous manifestations of: # Cerebrovascular disease (CVD) # Coronary artery disease (CAD) -Hypertension(HT) -Smoking -Diabetes(DM)
Examinations -Palpation of peripheral pulses (including abdominal aortic aneurysm(- Peripheral neuropathy
Investigations -Ankle-brachial pressure index -Blood pressure -Electrocardiography -Random blood glucose or HbA1c -Full blood count -Urea and electrolytes -Serum total cholesterol -Thrombophilia screen if patient <50 years old

Patients with asymptomatic claudication and those with claudication can both be treated in primary care settings (Table 2.2) (Morley, R. L. *et al.*, 2018).

Table 2.2. Patients with PAD in primary care (Morley, R. L. *et al.*, 2018).

All patients should receive the following before referral to secondary care.
Risk factor modification •HbA1c control (target value <48 mmol/mol) •Blood pressure control (target <140/90 mm Hg) • Smoking cessation therapy •Clopidogrel (or aspirin) 75 mg lifelong •Atorvastatin 80 mg† lifelong
Symptom control -Supervised exercise therapy for 3 months -Target blood pressure is for patients <80 years old. If >80 years, target is150/90 mm Hg. -Dose for secondary prevention of CVD.

2.1.4. Risk factors of Cardiovascular Disease

There is no known cause for CVD; however a variety of factors can contribute to its development. These factors are gender and age, as well as DM, HT, obesity, smoking, and inactivity (Mendis, S. *et al.*, 2011). The Iraqi Ministry of Health reported that 30% of Iraqis have HT, 14% have DM, and more than 35% are obese (Alwan, A., 2001). It has been discovered that 38% of Iraqi men smoke, 20% of men between the ages of 13 and 15, and 9% of women between the ages of 13 and 15 (Alwan, A., 2001).

2.1.4.1. Age and Gender

CVD has a high effect on aging. CVD affects about 13% of the Western population over age 50 (Morley, R. L. *et al.*, 2018). A study by Allender, S. *et al.* (2008) was reported that CVD is the main cause of death in Europe, accounting for 55% of deaths in women and 43% of deaths in men. CVD is the mostly cause of death in Europe before the age of 75 years, with about 44% of deaths in women and 38% of deaths in men under 75 years. Amen, S. O. *et al.* (2020) discovered that the majority of heart attack victims in Iraqi patients were men between the ages of 60 and 69. Comparatively, between the ages of 60 and 69, 39.1% of women and 46% of males who experienced an acute myocardial infarction (AMI) were over the age of 60. Acute MI was substantially more likely to occur in people under 60 than in people over 60. This indicates that a younger demographic is now making up the majority of those who present to the hospital with an acute MI. Compared to a few years ago, the average age of CVD patients in Iraq appears to have decreased (Alwan, A., 2001).

2.1.4.2. Dyslipidemia

Dyslipidemia is characterized by elevated TC, LDL, and TG values and decreased HDL levels. Blood plasma's primary TC carrier is LDL, and research has shown that TC considerably raises the chance of getting CVD. Reducing LDL levels can help patients avoid developing atherosclerotic disease. Low HDL levels have been associated with an increased risk of CVD, as well as an earlier onset of HD and a worse prognosis for those who already have it (Perk, J., 2009).

It is impossible to exaggerate the importance of lipid metabolism and variations in LDL in the emergence of atherosclerosis. In lipid metabolism, both exogenous and internal systems are involved. The exogenous route begins at this point. Chylomicrons start to be

produced and released by the intestine. Making TG-rich extremely LDL is the initial stage (VLDL particles). When fat is eliminated from the body, VLDL that isn't needed to store it is converted into LDL particles that carry TC and less TG (Wu, M. Y. *et al.*, 2017).

Patients who had an acute MI had high TC, high LDL, high TG and low HDL levels were more likely to have them than people who lived in Iran or the Middle East (Amen, S. O. *et al.*, 2020). The values of treatment should be assessed in patients with established CVD and those at high risk for developing CVD (Table 2.3).

Table 2.3. Lipid Goals for Patients at risk for Atherosclerotic CVD (Jellinger, P. S. *et al.*, 2017).

Lipid parameter	**Goal (mg/dL)**
Cholesterol	< 200
LDL	< 130 (low risk) <100 (moderate risk) <100 (high risk) <70 (very high risk) <55 (extreme risk)
Non-HDL TG	30 above LDL-C goal 25 above LDL-C goal <150
Apo B	<90 (patients at high risk of ASCVD, including those with diabetes <80 (patients at very high risk with established ASCVD or diabtes plus ≥1 additional risk factor) <70 (patients at extreme risk)
Apo : apolipoprotein; ASCVD : Atherosclerotic cardiovascular disease:HDL:High density lipoprotein cholesterol; LDL:Low density lipoprotein cholesterol; TG: Triglycerides	

2.1.4.3. Diabetes

High glucose levels are a sign of DM. They are more likely to get HD if their blood glucose levels are too high (Morley, R. L. *et al.*, 2018). More than 80% of deaths and 75% of hospitalizations in diabetic patients are caused by an acute CAD (Amen, S. O. *et al.*, 2020). It has been found that atherosclerotic plaques are more likely to rupture in diabetic people. DM causes atherosclerosis, which increased the risk of having a heart attack. It also has an effect on lipid profiles and encourages the growth of atherosclerotic plaque in the coronary arteries. So, it has been shown that MI in diabetic people is more deadly and severe than MI in people who are not diabetic. People with both types of DM have an incentive to keep their blood sugar levels in the best possible range. According to some research, better DM control can cut the risk of CVD by a lot (Amen, S. O. *et al.*, 2020). Glucose management in people with type 1 diabetes (T1DM) requires both the best insulin administration and professional nutrition therapy in the same way. Dietary counselling,

weight loss, and more physical activity should be the first line of treatment for people with T2DM, followed by pharmacological therapy (Perk, J., 2009).

2.1.4.4. Overweight or Obesity

Obesity is a complicated condition that causes a lot of fat to build up in the body, which can lead to other diseases like HD, DM, HT, and cancer. An index called the BMI is a rough measure of obesity in the general population. It's a person's weight divided by the square of height. BMI classification is given in table 2.4 (Consultation, W. H. O. 2000).

Table 2.4. BMI categories (Consultation, W. H. O. 2000)

BMI (category)	BMI (kg/(m)2
Underweight	Less than 18.5
Normal	18.5- 24.9
Overweight	25.0- 29.9
Obese	30-34.99
Morbidly obese	≥35

Multiple factors such as genetic, socioeconomic condition, lifestyles, and environtal are causes of obesity (Perk, J., 2009). The highest prevalence of overweight was found in those aged 50 to 59, while the highest prevalence of excess obesity was found in people aged 60 to 69. The prevalence of overweight was highest in female AMI patients, and the prevalence of excess obesity was highest in male AMI patients (Amen, S. O. *et al.*, 2020). For people who were overweight or obese, they had an abnormal lipid profile higher than for people who had a normal BMI (Amen, S. O. *et al.*, 2020).

2.1.4.5. Insulin Resistance

Issues with the absorption and use of glucose are the root cause of insulin resistance. Target cells experience IR when they don't react to regular insulin levels, necessitating the use of higher doses of the hormone. Both local and systemic IR has been associated with lipid build up, as well as the inhibition of glucose oxidation and adipocytokine release by fatty acid oxidation. The risk of HD may be reduced by treating IR (Ormazabal, V. *et al.*, 2018).

2.1.5. Prevention of Cardiovascular Diseases

Primary, secondary, and tertiary cardiovascular preventive techniques are the three different categories. The WHO report on CHD prevention was divided into three sections: general population strategies, high risk population strategies, and post-CHD initiatives. The population strategy aims to alter people's environments and way of life in order to more evenly distribute risk factors. to reduce the number of persons who contract the illness. The high-risk primary prevention strategy should be used by those who are healthy but have a very high risk of contracting the disease in the future. The secondary preventive approach is used to treat those who already have cardiac issues or damage. It has been demonstrated that these programs, which incorporate exercise instruction, enhance heart disease patients' care procedures, hospital readmission rates, functional status, and overall mortality (Perk, J., 2009). Table 2.5 lists the characteristics of people with healthy hearts, while Table 2.6 lists the preventive goal values for people with CVD.

Table 2.5. Features of a population with a heart- healthy (Perk, J., 2009).

No smoking
Healthy food choices
Physiccal activity; 30 min of moderate exercise a day
Body mass index of <25kg/m2 and to avoid central obesity
Blood pressure of <140/90 mmHg
Total cholesterol <5 mmol/l (190 mg/dl)
LDL-cholesterol<3 mmol/l (100 mg/dl)
Good glycemic control in diabetics

Table 2.6. Patients with documented CVD or DM, as well as people at high CVD risk, should have control limits (Perk, J., 2009).

Blood pressure of <130/80 mmHg
Total cholesterol <4.5 mmol/l (175 mg/dl), with an option of <4 mmol/l (155 mg/dl) if feasible
LDL-cholesterol<3 mmol/l (100 mg/dl), with an option of < 2.0 mmol/l (77 mg/dl) if feasible
Consideration of prophylactic drug therapy in particular groups, especially those with established atherosclerotic CVD

2.2. Salusin-α

New molecules called salusin-α are implicated in atherosclerosis. Blood flow is affected by salusin-α, which are bioactive peptides. They contribute to the occurrence of HD and atherosclerosis (Kolakowska, U. *et al.*, 2016). Preprosalusin, a 242-amino-acid precursor, is hypothesized to contain a C-terminal portion that is where salusin-α is formed. Preprosalusin is converted into prosalusin, which has 216 amino acids, by the removal of a signal peptide with 26 amino acids at its end. Salusin-α and Salusin-β, which have respective lengths of 28 and 20 amino acids, are synthesized via proteolysis at the C-terminus of prosalusin. Since their physicochemical characteristics aren't identical to those of salusin, there are more hydrophobic amino acid residues in salusin-β than salusin-α (Sato, K. *et al.*, 2013; Kolakowska, U. *et al.*, 2016).

2.2.1. The Role of Salusin-α in Cardiovascular Disease

Salusin-α may be the foundation of a test for anticipating atherosclerotic CVD. Additionally, medications based on salusin-α may be able to treat CVD and other illnesses (Sato, K. *et al.*, 2013).

Salusin-α prevents the development of foam cells in people by acyl-CoA-cholesterol acyltransferase-1 is down-regulated, which adds to the anti-atherosclerotic activity. Salusin-α has been proposed as a potential biomarker for HD and atherosclerosis (Fujimoto, K. *et al.*, 2013).

2.3. Salusin--β

When there are atherosclerotic plaques in the heart, salusins-β is released. Salusin-β expression is higher than salusin-α. During coronary artery bypass surgery in people with CAD, fibroblasts were found to make salusin-β in the vascular smooth muscle cells of the media layer and the media layer of the aorta. This was an in vivo study. Salusin-β stimulated growth of vascular smooth muscle cells and fibroblasts has been thought to play a role in atherosclerosis. It has a bad effect on health because of these effects (Sipahi, S. *et al.*, 2019; Kolakowska, U. *et al.*, 2016). Plasma salusin-β levels were significantly increased in those with CAD than in people without CAD, according to Sato, K. *et al.* (2013).

2.3.1. The Role of Salusin-β in Cardiovascular Disease

Salusin-β may be implicated in the genesis and pathophysiology of HT, according to Kolakowska, U. *et al.,* (2016)'s study. Salusin-β may also be related to atherosclerosis in both adolescents and adults. The posterior pituitary gland's ability to immediately reduce blood pressure and heart rate by producing vasopressin and oxytocin is assisted by salusin-β. Atherosclerosis, on the other hand, might be brought on by salusin-β in the vascular system. These findings reveal that salusin-α and salusin-β have opposing effects on atherosclerosis (Figure 2.5).

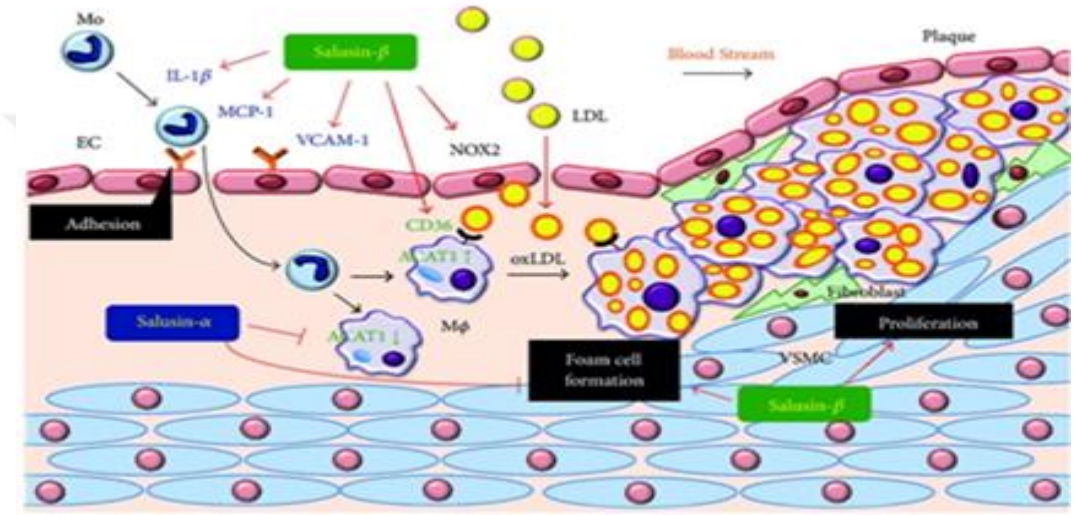


Figure 2.5. Modulatory effects of salusin-α and salusin-β on atherogenesis in vascular cells (Sato, K. *et al.*, 2013).

2.4. Irisin

It's a hormone produced by the breakdown of a protein called fibronectin type 3 domain-containing protein 5 (FNDC5), which would be found in muscle tissue, the cardiovascular, adipose cells, and the liver (Gizaw, M. *et al.*, 2017; Silvestrini, A. *et al.*, 2019). People with more irisin in their blood had better glucose homeostasis and less IR in the first study of this hormone. This is because irisin is made from its precursor, FNDC5. Irisin is a strong messenger that sends messages that help determine how each cell works (Gizaw, M. *et al.*, 2017). Irisin is a peptide-like hormone having a peptide structure made up of 112 amino acid residues. The three domain FNDC5 protein—fibronectin type III domain, hydrophobic C-terminal domain, and N-terminal signal domain—forms irisin via proteolytic cleavage at its carboxy terminus (Figure 2.6) (Gizaw, M. *et al.*, 2017).

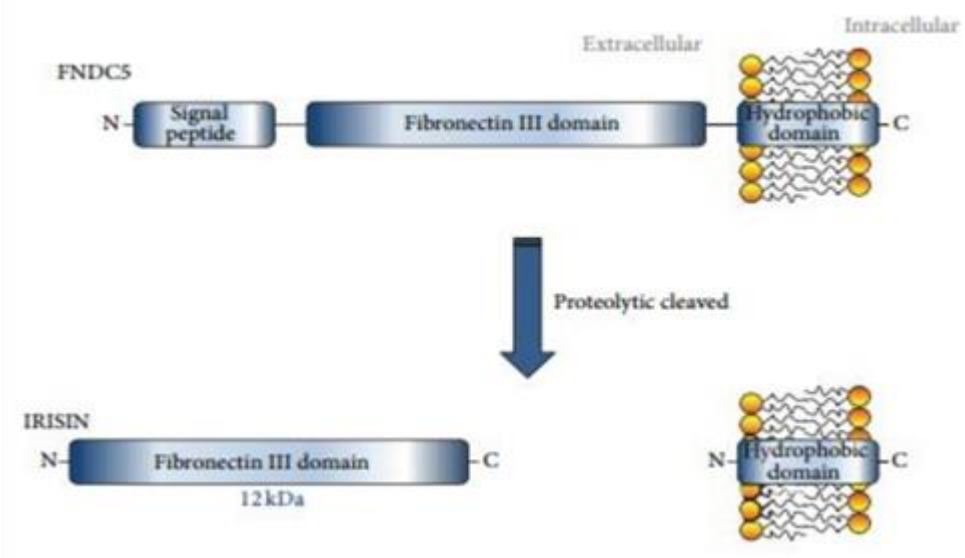


Figure 2.6. Structure of Irisin **(**Gizaw, M. *et al.*, 2017).

Since its discovering in 2012, it's been the subject of many studies because of its important role in the body. Irisin may be the key to understanding a lot of different illnesses and how they progress. The most interesting things about irisin are such impacts and potential application, but there is still a lot of debate about how irisin works, especially in terms of how it is expressed and how it interacts with receptors. This form of particle is releasing by heart as well as skeleton when people exercise. Irisin sends messages to other parts of the body, such as the heart, skeleton and parts of the body like the hepatic, fat, and brain. (Figure 2.7) (Gizaw, M. *et al.*, 2017).

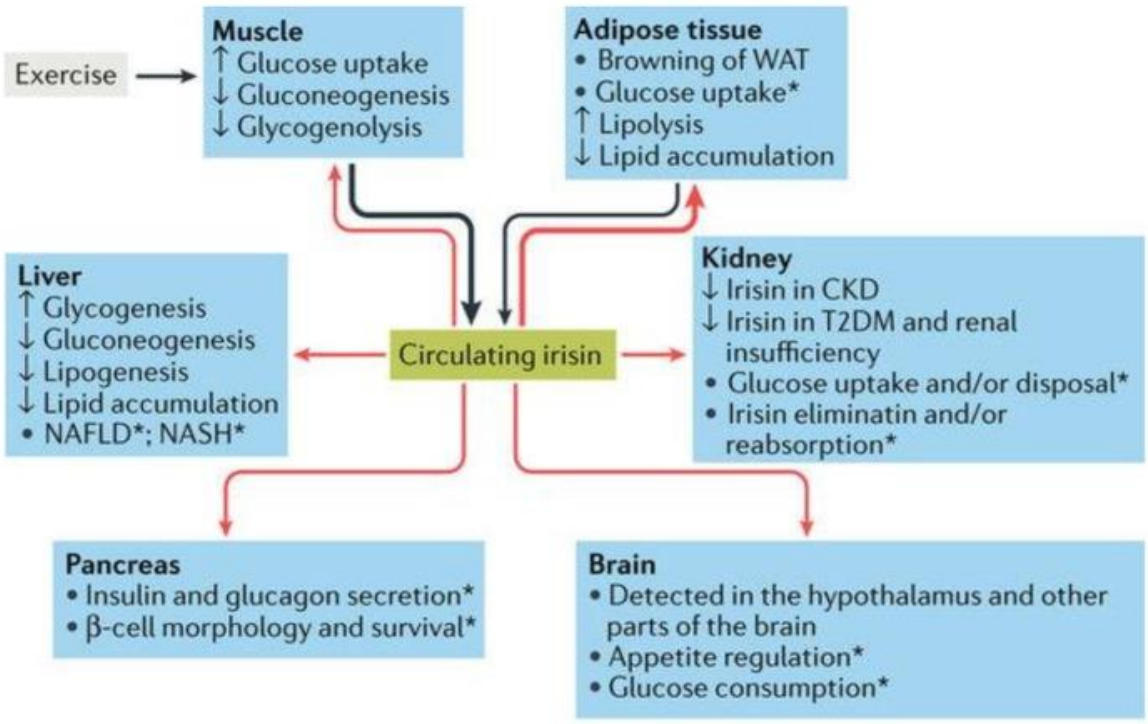


Figure 2.7. Potential roles of Irisin (Gizaw, M. *et al.*, 2017).

2.4.1. The Role of Irisin in Cardiovascular Disease

Since skeletal muscle produces less irisin than cardiac muscle does, FNDC5 is more prevalent in cardiac muscle. Circulating irisin levels continue to be associated with numerous forms of CVD, according to a substantial amount of research (Silvestrini, A. *et al.*, 2019). Irisin levels in T2DM patients were examined by El-Lebedy, D. H. *et al.* (2018)'s research revealed that CVD patients have lower Irisin levels than non-CVD patients and that there are close ties between HbA1c and TG levels in CVD patients. Hsieh, I. C. *et al.* (2018) examined the relationship between irisin levels and heart issues throughout the course of a three-year follow-up. The risk of CVD, a stroke, heart failure, or the requirement for revascularization increased as irisin levels increased four times more individuals (Pan, J. A. *et al.*, 2021).

3. MATERIALS AND METHODS

3.1. Subjects

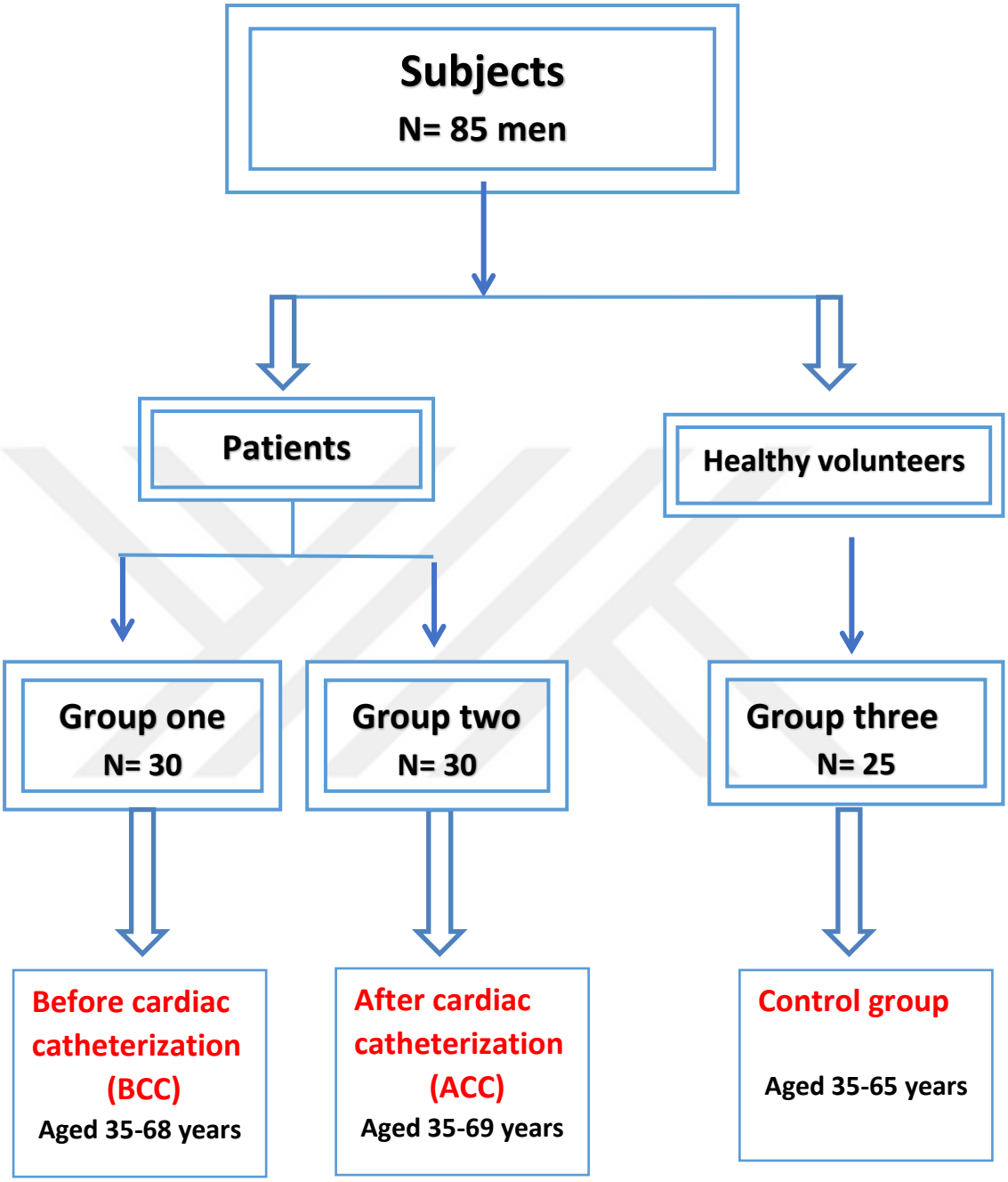
85 male individuals participated in the study. Before and after the subject underwent cardiac catheterization, serum samples were collected, and they were compared to serum samples from the healthy group.

The test was conducted on 30 patients before cardiac catheterization, 30 patients after cardiac catheterization, and 25 control groups with matched age and gender.

Between March 2021 and October 2021, patients were admitted to the Coronary Care Unit (CCU) from three hospitals in the Iraq-Anbar Governorate: Al-Ramadi Teaching Hospital, Ana General Hospital, and Rawa General Hospital. The purpose of this study was to assess the levels of salusin-α, salusin-β, irisin, lipid profile, glucose, insulin, and HOMA-IR in the blood of patients with cardiovascular disease (CVD) both before and after catheterization and compare those results with controls

All of the people in this study were investigated in the hospital by two senior cardiologists who both agreed that they had HF based on their clinical history, physical examination, lab results, and echocardiographic parameters.

3.1.1. Diagram of Study Samples



3.1.2. General Information

A questionnaire form called **Appendix 1** was filled out for each patient. It asked about their name, age, sex, HT, DM, personal history of CVD or atherosclerosis, lifestyle (smoking habits, and alcohol consumption), personal and family medical history. Whether the patient had taken drugs (to treat HF or lipid-lowering drugs or other diseases). A direct interview was used for all studied groups. The person with a BMI is a measurement by following the formula:

BMI = weight (Kg)/ height (m^2)

Whenever a person's body mass index (BMI) is really between 18.5 and 24.9, they are considered at their optimal levels of body weight, while being less than 18.5 indicates that the person seems to be underweight, a value ranging from 25 to 29.9 may reflect the fact that the individual is overweight, while ranging more than 30 may indicate that the individual is obese (Consultation, W. H. O. 2000).

The control group was made up of healthy people who didn't take medicines on a regular basis, didn't smoke or drink alcohol, and didn't have a known condition.

3.1.3. Exclusion Criteria

One of the most important criteria that were excluded from this study was the presence of any acute and chronic immune disorder, impaired liver activity, renal disease, various cancers, thyroid dysfunction, may be it has the potential to influence the path and outcome of the research.

3.2. Blood Sample Collection

Venepuncture samples taken by all patients and normal with the use of a 5 m plastic syringe, and the sample was then dispensed into a gel tubes and allowed to coagulate for 15minutes before being tested. After that, it was centrifugation at 3500 rpm for 10 minutes to obtain serum, which was then stored in the freezer (-20 °C) until it's used. The specimen was then divided in three parts:

1. The serum must have been stored at (80 °C) until it is needed for the estimation hormonal test in this study (salusin-α, salusin-β, irisin and insulin), were all measured in this section.

2. A section of the samples is kept at a temperature of 2-8 °C until the biochemical test, such as lipid analysis is performed.
3. Glucose levels are measured immediately after the blood is drawn.

Fasting blood samples of patients in group two were obtained after cardiac catheterization between (1- 6) months.

3.3. Materials

3.3.1. Reagents

3.3.1.1. ELISA Reagents

In this study all markers were assessment by using commercial enzyme linked immunosorbant assay (ELISA) microwells kits, which were utilized in accordance with the manufacturer's instructions.

3.3.1.1.1. Human Irisin ELISA Kit

The presence of irisin was determined using a human Irisin ELISA Kit; code YHB1765Hu, manufactured by SHANGHAI YEHUA Biological Technology Co., Ltd in China.

3.3.1.1.2. Salusin-α ELISA Kit

The amount of salusinα was determined by using a human salusin-α ELISA Kit; code YHB2627Hu, manufactured by SHANGHAI YEHUA biological technology Co., Ltd in China.

3.3.1.1.3. Salusin-β ELISA Kit

The amount of salusin-β was determined when using a human salusin-β ELISA Kit; code YHB2628Hu, manufactured by SHANGHAI YEHUA biological technology Co., Ltd in China.

3.3.1.1.4. Insulin ELISA Kit

The insulin level was determined by utilizing immunoassay analyzers (cobas c 111) are designed for use with the electrochemiluminescence immunoassay "ECLIA." REF 12017547122, originating in the US.

3.3.1.2. Reagents for Lipid Panel and Fasting Blood Glucose

The lipid profile (TC, TG, and HDL) and glucose levels were determined by cobas c 111 analyzer, only from the US.

3.3.2. Equipments

Table 3.1. Used equipment and manufacturers

Instrument	**Company**
Spectrophotometer	Cecil, Germany
Incubator	Fisher Scientific/ USA
Centrifuge	Hettich, Germany
Refrigerator with freezer -20C	LG, Korea
ELISA Microplate Reader	Genex Laboratories, Florida- USA
ELISA Microplate Washer	Genex Laboratories, Florida- USA
Cobas C 111 analyzer system	USA
Balance	Beurer/ Germany
Meter	Measuring 3M/ 10ft, China

3.4. Analysis

The ELISA method was used in this study to measure human hormones. This method is based on biotin double antibody sandwich technology. It's a simple and sensitive method.

3.4.1. Hormone ELISA Kit Assay

The Irisin, salusin-α, salusin-β, and Insulin concentrations were determined using the ELISA technique used in this study is competitive binding.

3.4.1.1. Measurement of Irisin

Test Principle

Biotin double antibody sandwich ELISA is used to test Human Irisin in this kit (Irisin). The monoclonal antibody Irisin has been pre-coated on the wells where Irisin is being incubated. Anti-irisin antibodies that have been labeled with biotin should be added after they have been incubated with streptavidin-HRP to make an immunological complex. After the enzymes have been incubated and washed, remove any enzymes that haven't bound to the substrates A and B. Addition of acid will make it turn blue and then yellow, Human Irisin concentration and the color of the solution are linked together.

Detection range: 0.05μg/mL→15μg/mL

Sensitivity：0.024μg/mL

3.4.1.2. Measurement of Salusin-α

Test principle

Human salusin (salusin-α), depend on ELISA because it is binding and depending on biotin double antibody sandwich technology. Incubate salusin (salusin-α) in wells that have been pre-coated with salusin (salusin-α) monoclonal antibody. After incubation, add biotin-labeled anti-salusin-α antibodies to combine with streptavidin-HRP to produce the immunological complex. After incubation and washing, remove any unbound enzymes before adding substrates A and B. With the addition of acid, the soluthion will converted from blue color to blue. The concentration of human salusin (salusin-α) and the color of the solution are positively associated.

Assay range：5 pg/mL→1000pg/mL.

Sensitivity：0.51 pg/mL.

3.4.1.3. Measurement of Salusin--β

Test principle

Human salusin (salusin-β), depend on ELISA because it is binding and depending on biotin double antibody sandwich technology. Incubate salusin (salusin-β) in wells that have been pre-coated with salusin (salusin-β) monoclonal antibody. After incubation, add biotin-labeled anti-salusin-β antibodies to combine with streptavidin-HRP to produce the immunological complex. After incubation and washing, remove any unbound enzymes before adding substrates A and B. With the addition of acid, the solution will converted from blue color to blue. The concentration of human salusin (salusin-β) and the color of the solution are positively associated.

Detection range: 10 pg/mL→1800 pg/mL.

Sensitivity：5.22 pg/mL.

3.4.1.4. Measurement of Insulin

Test principle

This test measures the interaction of human insulin by two monoclonal ab: one that has been fixed on wells containing plates and another that has been conjugated with (Insulin ELISA test) (HRP). Total duration of assay: 18 minutes. Followed by incubation, a straightforward steady washing procedure is used to remove the unbound substances and to complete the bound/free split. Once the bonded has been exposed to the H_2O_2 and TMB Substrate, the enzymes HRP in the connected interacts with them and produces a blue that changes to yellowish because when stopping solution (H_2SO_4) is introduced. The intensity of the color changes directly proportionate to the insulin levels in the specimen. A calibration graph is used to determine the insulin level in a sample before it is analyzed.

Detection range: 2.6‑24.9 µU/mL (17.8‑173 pmol/L)

Conversion factors: µU/mL x 6.945 = pmol/L

pmol/L x 0.144 = µU/mL

3.4.2. Biochemical Analysis

3.4.2.1. Glucose and lipid reagents

The lipid profile (TC, TG, and HDL) and glucose levels were determined only from use the cobas c 111 analyzer.

3.4.2.1.1. Measurement of Glucose

Principle of the test

The colorimetric reaction method occurs when hexokinase (HK) converts glucose to glucose-6 phosphate by catalyzes the phosphorylation (ATP).

$$Glucose + ATP \xrightarrow{HK} G-6P. + ADP$$

Proved this by using the enzyme glucose 6 phosphate dehydrogenase, which shows that glucose 6 phosphates can be converted to gluconate 6 phosphates when NADP is present. The appearance of color is caused by the rate at which NADPH is created, which is directly proportional to the amount of glucose present in the sample.

$$G6P + NADP \xleftrightarrow{G6PDH} gluconate - 6 - P + NADPH + H$$

Detection range: 74 – 109 mg/dl

4.11- 6.05 mmol/L

3.4.2.1.2. Measurement of Cholesterol

Principle of the tests

The cleave cholesterol ester function is used to liberate free cholesterol and fatty acids from cholesterol ester, a phospholipid. It is produced by the oxidation of cholesterol, which results in coolest-4-en-3-one and hydrogen peroxide, two products of the oxidation process. Final results showed that the oxidative coupling of 4-amino antipyrine with phenol resulted in forming a red quinone-mine dye when performed in the presence of peroxidase. The amount of TC contained in the sample has an inverse relationship with the intensity of the color.

Cholesteroi esters + H_2O $\xrightarrow{(CE.)}$ cholesterol RCOOH

Cholesterol + O_2 $\xrightarrow{(CHOD)}$ cholest-4-en-3-ono + H_2O_2

H2O2+ 4-aminotipyrine + phonol $\xrightarrow{(POD)}$ quinone-imine dye + 4H2O

Detection range: less than 200 mg/dL

< 5.2 mmol/L

3.4.2.1.3. Measurement of Triglyceride

Principle of the tests

Enzymatic colorimetric test; TG is breaks down to produce glycol and unsaturated FA, by using the LPL which can then be used to measure TC levels.

ATP phosphorylates Glycerol by glycerol kinase, resulting in the formation of Glycerol 3 phosphate (G-3-P) and adenosine 2 phosphates (ADP). G-3-P is converted to di-hydroxy-acetone phosphate and H_2O_2 by oxidation from the activity of the enzyme G-3-P -oxidase.

The 4- aminoantipyrine and phenol with H_2O_2 which facilitated by peroxidase, then formation the red chromophore that is dependent on the concentration of TG

Triglycerides $+3H_2O \xrightarrow{(Lipase)}$ Glycerol + 3 fatty acid

$$Glycerol + ATP \xrightarrow{Glycerol\ kinase} Glycerol - 3 - phosphate + ADP$$

Glycerol-3-phosphate + $O_2 \xrightarrow{(Glycerol\text{-}3\text{-}phosphate\ oxidase)}$ Dihydroxyaceton phosphate+ H_2O_2

$2H_2O_2$ + parachlorophenol + 4-aminoantipyrine $\xrightarrow{(Peroxidase)}$ Quinonemine+$4H_2O$

- Reference normal TG value < 150 mg/dL
- <1.70 mmol/L
- Conversion factors: mmol/L × 88.5= mg/dL

  mg/dL. × 0.0113= mmol/L

3.4.2.1.4. Measurement of HDL – Cholesterol

Principle of the tests

Non-HDL lipoproteins such as LDL, VLDL, and chylomicrons can be made water-soluble, allowing them to be eliminated from the body. After that, only HDL-particles are reacted with by CHER and CHOD. CHER and CHOD are enzymes that are involved in the measurement of HDL cholesterol levels as a result of CHER's enzymatic cleavage of cholesterol esters, free cholesterol and fatty acids are released into the environment.

HDL-cholesterol esters + $H_2O \xrightarrow{(DHEA)}$ HDL-cholesterol - RCOOH

In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to 4cholestenone and hydrogen peroxide.

HDL-cholesterol + $O_2 \xrightarrow{(CHOD)}$ 4cholestenone + H_2O_2

Antipyrine, EMSE, and 4-amino-antipyrine are all dyes formed when H_2O_2 is created by peroxidase reacts with the dye. The higher the concentration of TC, the brighter the color will be.

$2H_2O_2$ + 4–amino-antipyrine + EMSE+ H^{++} $H_2O \xrightarrow{(Peroxidase)}$colered pigment+$5H_2O$

$2H_2O_2$+4–amino-antipyrine + EMSE + H^{++} $H_2O \xrightarrow{(Peroxidase)}$ colered pigment+$5H_2O$

Apo lipoprotein B – containing lipoproteins (VLDL, HDL) is chemically with phosphotungsticacid/magnesium chloride, centrifugation of the precipitant agent and enzymatic analysis of HDL as residual TC remaining in the supernatant.

- Reference normal HDL (Females) value ≥ 65 mg /dL
- >1.68 mmol/L
- Reference normal HDL (males) value ≥ 55 mg /dL
- >1.45 mmol/L
- Conversion factors: mmol/L × 38.66 = mg/dL

mg/dL. × 0.0259= mmol/L

3.4.2.1.5. Measurement of LDL– Cholesterol

Test principle

LDL cholesterol esters and free cholesterol are measured utilizing homogeneous enzymatic colorimetric tests in which cholesterol esterase and cholesterol oxidase are utilized in the presence of surfactants that solely solubilize LDL cholesterol esters and free cholesterol. Surfactants and a sugar molecule interfere with the enzyme's reactions to lipoproteins other than low-density lipoprotein (LDL).

LDL-cholesterol esters + H_2O $\xrightarrow{\text{(Cholesterol esteras)}}$ cholesterol + free fatty acids

TC concentrations in HDL, VLDL, and chylomicrons are yet unclear.

When cholesterol esters are broken apart, the enzyme cholesterol esterase destroys them, releasing free cholesterol fatty acids into the bloodstream.

LDL-cholesterol + O_2 $\xrightarrow{\text{(Cholesterol oxidase)}}$ 4cholestanone + H_2O_2

The enzyme cholesterol oxidase When oxygen is present, 4-cholestenone and hydrogen peroxide oxidizes TC.

2 H_2O_2+ 4-aminoantipynino + EMSE + H,0 + H $\xrightarrow{\text{(DHEA)}}$ red purple pigment + 5 H_2O

Antipyrine, EMSE, and 4-amino-antipyrine are all dyes formed when H_2O_2 is created by peroxidase reacts with the dye. The higher the concentration of TC, the brighter the color will be.

Expected values

Level in terms of risk for CVD.

Adult levels:

Optimal.	<2.59 mmo/L (< 100 mg/dL)
Near optimal/above optimal	2.59-3.34 mmo/L (100-129 mg/dL)
Borderline high.	3.37-4.12 mmo/L (130-159 mg/dL)
High.	4.14-4.89 mmo/L (160-189 mg/dL)
Very high	≥ 4.92 mmol/L (≥ 190 mg/dL)

3.4.2.1.6. Measurments of VLDL– Cholesterol

The levels of VLDL exist in serum equal to one fifth or 0.2 of the TG concentration. The level of serum of VLDL was calculated as follows:

VLDL= 0.2 × Triglyceride

3.4.3. Homeostatic Model Assessment (HOMA-IR)

A (HOMA-IR) is known as the Homeostasis Model Assessment Insulin Resistance that calculates was used to estimate insulin sensitivity in this study. The formula is as follows:

HOMA-IR = Fasting insulin × fasting glucose/ 405

(This is applied when glucose measured in mg/dl and where is fasting plasma insulin concentration (µU/L) (Matthews, D. R., *et al.*, 1985).

HOMA-IR = Fasting insulin × fasting glucose/22.5

(This is applied when glucose measured in molar unit's mmol/L and insulin in pmol/L) (da Silva, R. C. Q. *et al.*, 2007).

The HOMA-IR cut-off point was determined to be 2.6 for adults. HOMA-IR<2.60 as the normal range, HOMA-IR ≥2.60 as having IR (McAuley, K. A. *et al.*, 2001), Fasting insulin level ≥12μU/l was considered as IR among both non-diabetic and diabetic populations (Majid, H. *et al.*, 2017).

3.5. Statistical Analysis

All data were analyzed for normality, homogeneity, and normally distributed. The student t-test used to assessment the magnitude of the correlation between the average scores of any two groups. The statistical analyses are analyzed using Microsoft Excel 2013 and **SPSS-22**. There is a mean and a standard deviation for the data reported (SD). Pearson's correlation coefficient is employed to determine correlation, and statistical significance is determined as $p<0.05$.

4. RESULTS

4.1. The Study's Population Demographics

In the Al-Anbar/Iraq governorate, a cross-sectional hospital-based study was done to determine if salusin α -, salusin- β, and irisin levels in patient populations during (before and after) cardiac catheterization are linked. To determine if there was a difference between the parameters and the patient group included in the research, a comparison analysis was undertaken. An attempt was made to find a relationship between all factors. The study group was then separated into over 50 and under 50 year old groups, as well as obese, overweight, and non-obese groups, to check whether there was a ratio difference across subgroups and to understand how age and obesity influenced all metrics in this study.

There were 85 Iraqi patients who participated in this research, who were divided into three groups: 30 patients who underwent before cardiac catheterization (BCC group), 30 patients who underwent after cardiac catheterization (ACC group), and the remaining group serving as a control group (25 patients). Serum salusin-α, salusin-β, irisin, insulin, glucose, TC, total TG, HDL, and LDL are all measured. The mean and standard deviation of each parameter were calculated. The patients' serum before and after cardiac catheterization is collected and compared with that of the control group and compared between them. In addition to comparing each parameter with subgroup of age and BMI.

Doctors used the term "CVD" to describe patients who had a stroke, heart attack, congestive HF, or used coronary vasodilators or antianginal. T2DM was diagnosis and defined by a physician as a fasting glucose concentration of more than or equal to 7 mmol/L, or the use of blood glucose-lowering medicines. Dyslipidaemia is defined when there are high levels of TG, TC, LDL and low levels of HDL or use the dyslipidaemia medicines. All of the people who took part in the study were put into groups based on their age (Age <50 years and Age ≥50 years) and BMI [18.5–24.9 kg/m^2; normal weight], [25–29.9 kg/m2; overweight**)**], and obese [≥30 (kg/m^2**)**].

4.2. Cardiovascular Disease Relation to Age

The control group were average age was between 35 and 60 years old, with age of mean of 42.3±4.3 years old. The BCC group had a mean of 53.65 ±2.5 years and a range of 35-68 years. The ACC group had a mean age of 55.72±2.5 years and a range of 35-69 years (Table 4.1). In this study, we split the age of patients who underwent cardiac catheterization into two groups (those under 50 and those over 50). The findings revealed, there was a statistically significant difference ($p<0.05p$) of age in BCC and ACC group (53.65± 2.5 years and 55.72±2.5 years) in comparison with control group (42±4.3 years). Additionally, the results of the tests demonstrated that the number of catheterization patients in the age group more than 50 years was higher than the number of catheterization patients in the age group less than 50 years. [BCC patients 58.6± 2.1years and 42.1± 3.8; ACC patients; 60.8± 1.55 years and 44.53± 1.3; control group; 60.43±2.9 years and 38.8± 4.2; $p<0.05$] as shown in Table 4.1

Table 4.1. Average age and prevalence: N (%) of sample for patients with cardiovascular disease groups and control group

Groups		N (%)	Age (years) mean ± SD	Significant *p*- value
Before cardiac catheterization BCC	Age <50	11 (36.7%)	42.12 ± 3.8	*t* = 7.733 *p < 0.0001*
	Age ≥50	19 (63.3%)	58.60± 2.1	
	Total	30(100%)	53.65± 2.5	
After cardiac Catheterization ACC	Age <50	9(30%)	44.53± 1.3	*t* =8.733 *p*< 0.0001
	Age ≥50	21(70%)	60.8± 1.55	
	Total	30(100%)	55.72±2.5	
Control groups	Age <50	17(68%)	38.8± 4.2	*t*= 13.735 *p*<0.001
	Age ≥50	8(32%)	60.43±2.9	
	Total	25(100%)	42± 4.3	
Chi square= 39.4		*p*-value <0.0001		

t: t-test statistic. *p*: Two-tailed probability, N:Number of patients,SD: standard deviation

4.3. Body Mass Index (BMI)

The BMI was divided into three groups in this study according to Consultation, W. H. O. (2000). Table 4.2, demonstrates the distribution of BMI of control, BCC and ACC groups. The numbers of non-obese, overweight, and obese in control group were 12(48%),

10(40%) and 3(12%) respectively whereas in BCC-and ACC patients they were 5 (16.7%), 10(33.3%), 15 (50%) and 6(20%), 11(36.7%), 13 (43.3%) respectively.

Table 4.2. The mean±SD of BMI for BCC, ACC patients and control groups

Groups					
Parameters			**Control group N=25**	**Before Catheterization BCC group (N= 30)**	**After Catheterization ACC group (N= 30)**
BMI (Kg/m²)	≤24.9	mean ± SD	23.19±1.5	23.56±1.03	23.34±1.33
		No.	12(48%)	5 (16.7%)	6(20%)
	25-29.9	mean ± SD	27.39±0.87	26.75±1.024	27.15±1.06
		No.	10(40%)	10(33.3%)	11(36.7%)
	≥30	mean ± SD	31.7±2.5	32.90±2.05	32.32±1.77
		No.	3(12%)	15 (50%)	13 (43.3%)
Chi square = 16.3 ***p***-value:0.004					
N: Number of patients.					

4.3.1. BMI Levels

The BMI for each group were determined and were displayed in table .4.3. The results indicated there was a significant (p<0.05p) increase in BMI in the BCC (30.5 ± 3.96 kg/m^2) and ACC groups (29.09±3.66 kg/m^2) as compared to the control group (24.9±2.2kg/m^2), Additionally, the results indicated that there's no significant difference (p>0.05p) between the BCC and ACC groups.

Table 4.3. The Mean± SD of BMI for individuals with BCC, ACC, and the control group.

Groups / Parameter	Before Catheterization BCC group (N= 30)	After Catheterization ACC group (N= 30)	Control group (N= 25)
BMI (kg/m^2) mean±SD	30.5 ± 3.96 a	29.09±3.66 a	24.9±2.2 b

Similar letters mean that there are no significant differences between them ($p> 0.05$). Different letters mean that there are significant differences between them ($p< 0.05$). SD:Standard deviation, N: Number of patients.

4.4. Salusins-α Levels

Salusins-α were measured in the serum of all participants. The Table 4.4 shows the mean of salusins-α for each of group. The results from the analysis showed that there have been a significant (p<0.05p) decrease of salusins-α value in BCC patents (81.06±0.72 pg/mL) and in ACC patients (110.63±0.47 pg/mL) comparison with control (168.2±0.445 pg/mL). Additionally, the results indicated a significant (p<0.05p) decrease in BCC patients when compared with ACC patients groups.

Table 4.4. The Mean± SD of salusin-α levels for BCC, ACC patients with control group as correlation with BMI

Groups / Parameter	Before Catheterization BCC (N=30)	After Catheterization ACC (N=30)	Control group (N=25)
Salusin-α pg/mL mean ± SD	81.06±0.72 a	110.63±0.47 b	168.20±0.445 c

Similar letters mean that there are no significant differences between them ($p> 0.05$). Different letters mean that there are significant differences between them ($p< 0.05$). SD:Standard deviation, N: Number of patients.

4.5. Salusin-β Levels

Mean serum concentration of salusin-β was shown in the Table 4.5. The mean of salusin-β in BCC (356.133±0.63 pg/mL) and ACC patients group (293.93±0.66 pg/mL) were higher

than the mean of control subjects (205.08±0.47 pg/mL). Significant correlation was ($p< 0.05$) in these groups.

The results in the Table 4.5 also was shown no significant ($p>0.05$) in salusin-β levels for BCC group (356.13±0.63 pg/mL), in comparison to ACC group (293.93±0.66 pg/ml).

Table 4.5. The mean±SD of Salusin-β levels for all groups

Groups / Parameter	Before Catheterization BCC (N=30)	After Catheterization ACC (N=30)	Control groups (N=25)
Salusin-β pg/mL mean ± SD	356.13±0.63 a	293.93±0.66 a	205.08±0.47 b

Similar letters mean that there are no significant differences between them ($p> 0.05$). Different letters mean that there are significant differences between them ($p< 0.05$). SD:Standard deviation, N: Number of patients.

The ROC curve analysis indicated the good performance of salusin-α and salusin-β in diagnosis (Figure 4.1). For cut-off point of salusin-α is 95, sensitivity is 97.8 % and specificity 97.4%, AUC 0.997 (95% CI 0.952–1.000). Similar results have been reported in the cut-off point of salusin-β is 214, sensitivity is 100 % and specificity 95.2%, AUC 0.997 (95% CI 0.948 to 1.000).

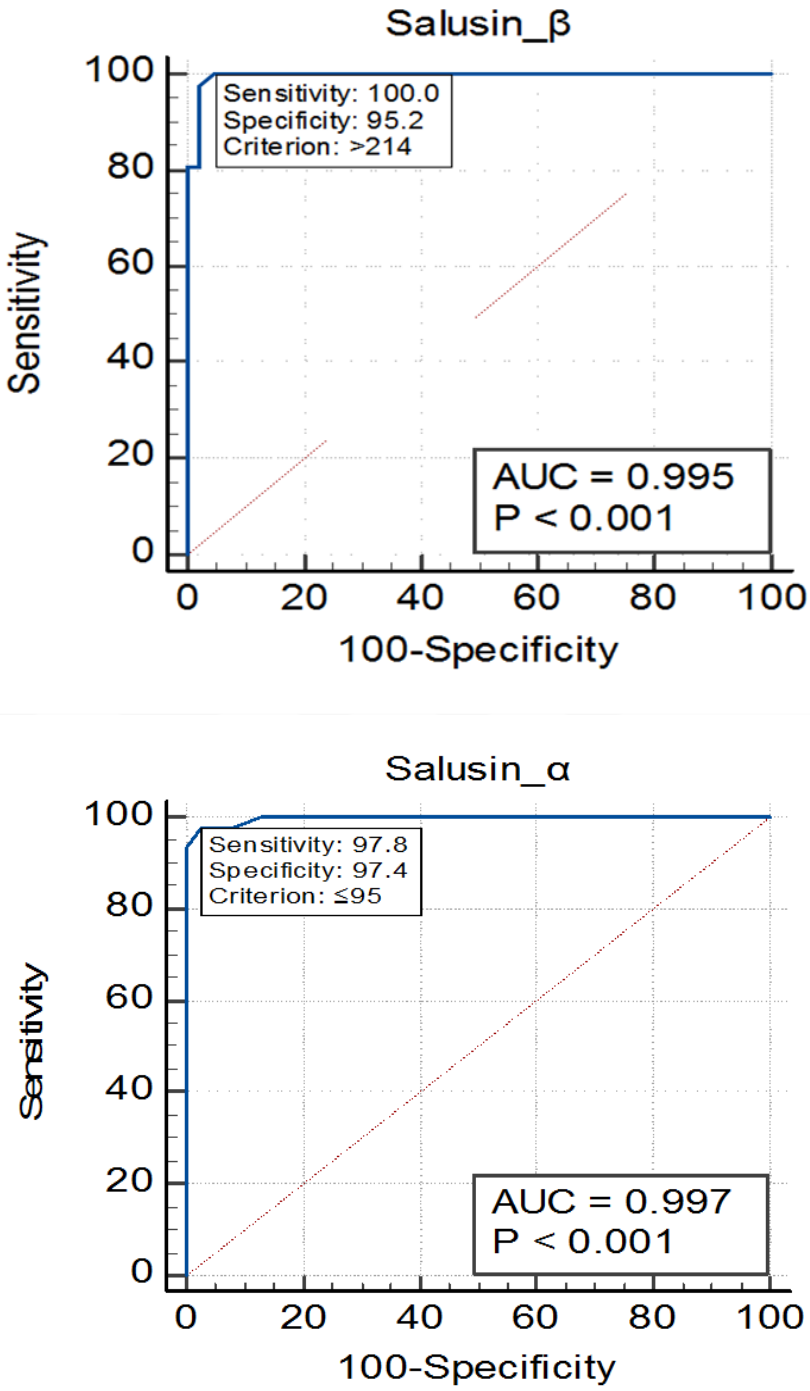


Figure 4.1. The ROC curve analysis of the performance of salusin-α and salusin-β.

4.6.The Relation between Serum Salusin-α with Age and BMI

Table 4.6. and figures in **(Apendix 2)** observed that the serum salusin-α levels in human gradually decline with increasing of BMI and age in BCC and ACC patients (*p> 0.05*)., but not in control (*p> 0.05*) (may be due to the most of the patients in this group were under the age of 50 and non-obese).

Table 4.6. The mean±SD of Salusin-α level for all groups in relation to age and BMI

Groups / Parameter	Sub-groups	Salusin-α concentration pg/mL		
		Before Catheterization BCC group (N= 30) BC	After Catheterization ACC group (N= 30)	Control groups (N=25)
BMI (Kg/m²) Mean±SD	**≤24.9**	88.4±0.82) a	181.66±0.30 b	164.50±0.51 b
	25-29.9	84.167±0.75 a	102.26±0.46 a	172.66±0.41 b
	≥30	53.235±0.35 c	103.19±0.45 a	170.74±0.60 b
Age years Mean±SD	**Age <50**	97.10±0.70 a	113.11±0.59 a	166.31±0.49 b
	Age ≥50	73.05±0.72 a	110.01±0.42 a	174.16±0.30 b

Similar letters mean that there are no significant differences between them (*p> 0.05*). Different letters mean that there are significant differences between them (*p< 0.05*). SD:Standard deviation, N: Number of patients

4.7. The Relation between Serum Salusin-β with Age and BMI

Salusin-β levels in humans increased in people who are more than 50 years old than less 50 years old (*p> 0.05*). The Salusin-β levels were increased with increased obesity during BCC and ACC (*p< 0.05*)., but not increased in control group (*p> 0.05*). (It is possible that this was related to the majority of patients in this group was under the age of 50 and was not obese), as shown in Table 4.7. and Appendix 3.

Table 4.7. The mean±SD of Salusin-β levels for all groups in relation to age and BMI.

Groups / Parameter	Sub-groups	Salusin-β concentration		
		Before catheterization BCC group (N=30)	After Catheterization ACC group (N=30)	Control groups (N=25)
BMI (Kg/m²) Mean±SD	**≤24.9**	140±0.34 a	210.3±0.24 a	223.0±0.50 a
	25-29.9	333.9±0.66 c	209.5±0.47 a	188.2±0.39 a
	≥30	441.3±0.583 b	368.8±0.63 c	200.4±0.83 a
Age years Mean±SD	**Age <50**	295.0±0.78 a	268.1±0.88 a	223.5±0.43 a
	Age ≥50	386.7±0.57 b	287.8±0.62 a	246.5±0.56 a

Similar letters mean that there are no significant differences between them ($p > 0.05$). Different letters mean that there are significant differences between them ($p < 0.05$). SD:Standard deviation, N: Number of patients.

4.8. Serum Irisin Level

Irisin level (1.24±0.48 µg/mL) in BCC patients has been a statistically ($p < 0.0001$) lower than the mean control (2.51±0.46 µg/mL) as shown in the Table 4.8. Also there was a significant ($p = 0.0021$) decrease in serum irisin concentration for ACC patients group (1.530±0.771 µg/mL) in comparison to healthy control samples. Patients with BCC group had lower levels of irisin in their blood than patients with ACC group ($p = 0.0127$), as shown in Table 4.8.

Table 4.8. The mean±SD of Irsin level for all groups**.**

Groups / Parameter	**Before Cardiac Catheterization BCC (N=30)**	**After Cardiac Catheterization ACC (n=30)**	**Control groups (N=25)**
Irisin µg/mL mean ± SD	1.242±0.48 a	1.530±0.771 b	2.516±0.46 c

Similar letters mean that there are no significant differences between them ($p > 0.05$). Different letters mean that there are significant differences between them ($p < 0.05$). SD:Standard deviation, N: Number of patients.

The statistical analysis of our results showed the ROC curve analysis in Figure 4.2 indicated irsin a good diagnosis in CVD patients and cut-off value of irisin 1.3, sensitivity is 85.71% and specificity 97.4%, AUC 0.997 (95% CI 0.952–1.000).

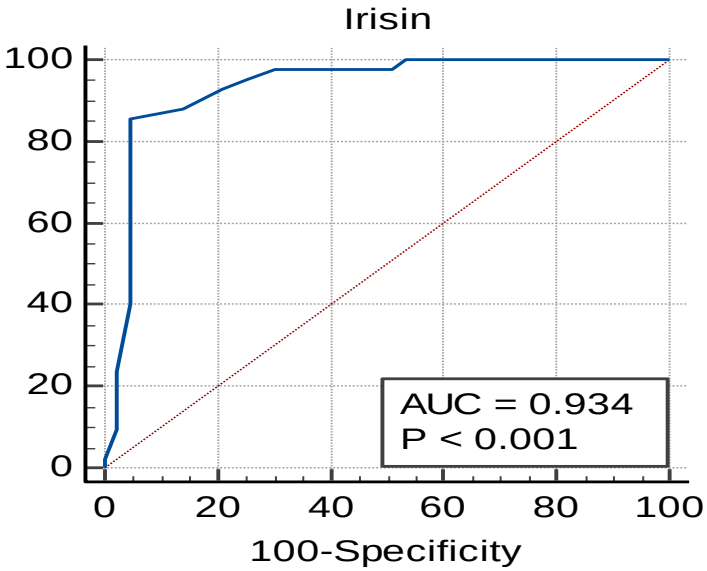


Figure 4.2. The ROC curve analysis of the performance of Irisin

4.9. The Relation between Serum Irisin Levels with Age and BMI

Findings showed the relation between serum irisin with age and BMI in studied groups (Tuple 4.9) and figure in (Appendix 3). The mean serum irisin level in normal, overweight and obese groups in BCC group was lower than the mean of ACC group patients and control group subjects as shown in Table 4.9. Also the results point out the cardiac catheterization patients in age group ≥ 50 years was higher levels of irisin than those recorded in age group < 50 years in ACC patients and control group but no significant differences was found *(p> 0.05)*. While serum irisin levels lower in aging (≥ 50 years) than in aging (< 50 years) in BCC patients (p = 0.052).

Table 4.9. The mean±SD of Irsin levels for BCC, ACC patients and control group in relation to age and BMI

Groups / Parameter	Sub-groups	Irisin concentration µg/ml		
		Before Cardiac Catheterization BCC (n=30)	After Cardiac Catheterization ACC (n=30)	Control groups (n=25)
BMI (Kg/m^2) mean ± SD	**≤24.9**	0.73±0.20 a	1.48±0.44 b	2.78±0.48 c
	25-29.9	1.10±0.475 a	2.03±0.72 b	2.12±0.32 b
	≥30	0.97±0.223 a	1.06±0.44 a	2.93±0.52 c
Age Years mean ± SD	**Age <50**	1.54±0.45 a	1.07±0.36 b	2.45±0.48 c
	Age ≥50	1.09±0.42 a	1.64±0.72 b	2.71±0.42 c

Similar letters mean that there are no significant differences between them ($p > 0.05$). Different letters mean that there are significant differences between them ($p < 0.05$). SD:Standard deviation, N: Number of patients.

4.10. Lipid Profile

When compared to the control group, the BCC and ACC groups had substantially ($p < 0.05$) greater levels of blood TC, LDL, TG, and VLDL, with a reduction in HDL (Table 4.10). Furthermore, substantial ($p < 0.05$) reductions in serum TC, LDL, TG, VLDL, and HDL levels were detected in the ACC group as compared to the BCC group.

Table 4.10. The mean±SD of lipid profile level for all groups.

Parameter / Groups	S.TC mean ± SD	S.TG mean±SD	S.LDL mean±SD	S.H.D.L mean±SD	S.VLDL mean±SD
Before catheraization BCC group	210 ±68 a	228±92 a	133±61 a	37±9.8 a	45±18 a
After catheraization ACC group	188±42 b	168±47 b	104±42 b	46±10 b	39±21 b
Control group	163±27 c	125±43 c	82±24 c	59±11 c	28±12 c
***p*-value**	*p< 0.05*	*p< 0.05*	*p< 0.05*	*p< 0.05*	*p< 0.05*

Different letters mean that there are significant differences between them ($p < 0.05$). SD:Standard deviation.

4.11. Serum Glucose Level

The mean serum glucose level in before catheterization patients (BCC: 123.3±0.6mg/dl) and after catheterization (ACC: 110.867± 0.467) was statistically significant (p= 0.026 and p= 0.0080 respectively) higher than the mean of control group (85.680±0.082 mg/dl). As well as, the results indicated that there's no significant difference (p= *0.469*) between the BCC and ACC groups. as shown in Table 4.11.

Table 4.11. The mean±SD of serum glucose level for all groups.

Groups / Parameter	Before Catheterization BCC **N=30( )**	After Catheterization ACC **(N=30)**	**Control groups (N=25)**
glucose mean ± SD	123.2±0.66 a	110.8± 0.467 a	85.6±0.082 b

Similar letters mean that there are no significant differences between them (p> *0.05*). Different letters mean that there are significant differences between them (p< *0.05*). SD:Standard deviation, N: Number of patients.

Serum glucose levels were likewise considerably greater with increasing BMI, according to Table 4.12. We discovered that glucose levels were greater in the group over 50 years old than in the group under 50 years old.

Table 4.12. The mean±SD of Glucose levels for all groups in relation to age and BMI.

Groups / **Parameter**	Sub-groups	Glucose levels mean ± SD		
		Before Catheterization BCC N=30	After Catheterization ACC N=30	Control groups (N=25)
BMI (Kg/m^2) mean ± SD	**≤24.9**	79±0.25 a	72±0.233 a	84±0.077 a
	25-29.9	135±0.63 c	110±0.40 b	90±0.97 a
	≥30	146±0.619 c	120±0.61 b	113±1.2 b
Age Years mean ± SD	**Age <50**	98±0.60 a	98±0.32 a	84±0.05 a
	Age ≥50	135±0.65 b	114±0.42 a	85±0.09 a

Similar letters mean that there are no significant differences between them (p> *0.05*). Different letters mean that there are significant differences between them (p< *0.05*). SD:Standard deviation, N: Number of patients.

4.12. Serum Insulin Level

The mean and standard deviation of insulin for each of the study groups is shown (Table 4.13). In both BCC and ACC groups (15.2± 0.46 ng/mL and 12.4± 0.57 ng/mL) respectively, insulin levels are considerably significant (p<0.05) greater than those in the control group (5.709±2.24 ng/mL). Also the ACC group had lower insulin levels than BCC group and no significant difference (P>0.05) was found.

Table 4.13. The mean±SD of insulin levels for BCC, ACC patients and control group

Groups / **Parameter**	Before Catheterization BCC **(N=30)**	After Catheterization ACC **(N=30)**	Control groups **(N=25)**
Insulin mean ± SD	15.2±0.46 a	12.4±0.57 a	5.7±2.24 b

Similar letters mean that there are no significant differences between them ($p> 0.05$). Different letters mean that there are significant differences between them ($p< 0.05$). SD: Standard deviation, N: Number of patients.

It is shown in table 4.14. and appendix 6 that insulin levels were increased with increasing obesity and age.

Table 4.14. The mean±SD of Insulin levels for all groups in relation to age and BMI

Groups / **Parameter**	Sub-groups	Insulin levels mean ± SD		
		Before catheterization BCC (N=30)	After Catheterization ACC (N=30)	Control groups (N=25)
BMI (Kg/m^2) mean ± SD	**≤24.9**	8.0±0.29 a	6.6± 0.19 a	4.9±0.38 a
	25-29.9	14.7±0.49 b	12.7±0.67 b	6.9±0.39 a
	≥30	16.8±0.44 b	13.5±0.46 b	6.8±0.39 a
Age Years mean ± SD	**Age <50**	11.8±0.57 a	10.1±0.62 a	5.2±0.40 b
	Age ≥50	16.9±0.40 a	12.0±0.57 a	7.1±0.41 b

Similar letters mean that there are no significant differences between them ($p> 0.05$). Different letters mean that there are significant differences between them ($p< 0.05$). SD: Standard deviation, N: Number of patients.

4.13. Homeostasis Model Assessment of Insulin Resistance (HOMA-IR)

Mean serum concentration of HOMA-IR as shown in Table 4.15. The mean of HOMA-IR were higher in the study group patients (BCC and ACC) than the mean of control subjects group. Significant ($p< 0.05$) difference was observed between them. Interestingly, a strong correlation between HOMA-IR and risk to develop CVD has been established.

The results in Table 4.15 also revealed a non- significant ($p>0.05$) increase of HOMA-IR for BCC group when compression with ACC group.

Table 4.15. The mean±SD of HOMA-IR levels for BCC, ACC patients and control group

Groups / **Parameter**	Before Catheterization BCC **(N=30)**	After Catheterization ACC **(N=30)**	**Control groups (N=25)**
HOMA-IR mean ± SD	4.5±0.63 a	3.5±0.85 a	1.2±0.46 b

Similar letters mean that there are no significant differences between them ($p> 0.05$). Different letters mean that there are significant differences between them ($p< 0.05$). SD:Standard deviation, N: Number of patients.

Table 4.16 and Appendix 7 indicate the mean SD of HOMA-IR values for BCC, ACC, and control patients in relation to age and BMI. IR levels rise considerably with obesity and age groupings. These findings indicate a positive significant connection between HOMA-IR and CVD risk in all research groups when compared to BMI and age.

Table 4.16. The mean±SD of HOMA-IR levels for all groupsin relation to age and BMI

Groups / **Parameter**	Sub-groups	**HOMA-IR mean ± SD**		
		Before Catheterization BCC (N=30)	After Catheterization ACC (N=30)	Control groups (N=25)
BMI (Kg/m²) mean ± SD	**≤24.9**	1.34±0.41 a	1.31 ±0.150 a	1.02±0.42 a
	25-29.9	3.43±0.85 b	3.52±1.04 b	1.50±0.42 a
	≥30	6.03± 0.67 c	4.04± 0.67 b	1.9±0.51 a
Age Years mean ± SD	**Age <50**	3.01±0.88 a	2.083±1.18 b	1.128±0.46 b
	Age ≥50	5.31±0.51 b	3.81±0.80 c	1.50±0.44 b

5. DISCUSSION AND CONCLUSION

Patients who had cardiac catheterization were separated into two groups based on their age in Table 4.1. (Under 50 years old and over 50 years old). The data revealed that the number of catheterization patients over 50 was greater than the number of catheterization patients under 50. People with cardiac difficulties were found to be older than those without as part of the present investigation. The findings are consistent with the findings of Allender, S. *et al.* (2008), who showed that in Europe, around 1.88 million persons die from CVD before the age of 75 each year. CVD is responsible for 44% of deaths in women under the age of 75 and 38% of fatalities in males under the age of 75. The majority of CVD patients in Iraq are followed at a younger age, with a mean age of 54 years across all research groups Table 4.1. In terms of age, the implications may be attributable to patients in Iraq not getting adequate physical exercise.

Obesity, high blood pressure, dyslipidemia, smoking, and diabetes are all risk factors. This conclusion was consistent with the findings of Amen, S. O. *et al.* (2020), who investigated the prevalence of AMI and the most frequent risk factors in Iraqi patients. The majority of male patients with acute MI were found to be between the ages of 60 and 69. This suggests that the average age of CVD patients admitted to hospitals is decreasing. Obesity is linked to a higher risk of death from all causes and to cardio metabolic risk factors. People who are overweight or obese, on the other hand, have a better chance of living longer especially if they have CVD (Lin, G. M. *et al.*, 2016). The BMI was divided into three groups in this study (Consultation, W. H. O. 2000). Table 4.2 demonstrates distribution of BMI of control and BCC and ACC patients group. The numbers of non-obese, overweight, and obese in control group were 12(48%), 10(40%) and 3(12%) respectively whereas in BCC- and ACC patients they were 5 (16.7%), 10(33.3%), 15 (50%) and 6(20%), 11(36.7%), 13 (43.3%) respectively.

The BMI for each group was calculated and is given in Table 4.3. The results revealed a substantial (p<0.05) rise in BMI in the BCC and ACC groups as compared to the control group, but no significant difference (p>0.05) between the BCC and ACC groups. According to the findings of Cha, Y. M. *et al.* (2008), 44 % receiving catheter ablation for

atrial fibrillation were overweight, and roughly 40 percent of patients were obese. Canning, K. L. *et al.* (2014) found the same findings (2014). People were young and middle-aged, according to the authors, and BMI was also a major predictor of CVD, high TC, and T2DM. As people become older, the association between BMI and metabolic problems becomes stronger. Several studies have found that obesity causes too many changes in the cardiovascular anatomy, which leads to the process of atherosclerosis (Bastien, M. *et al.*, 2014). Cha, Y. M. *et al.* (2008) found that 65 percent of American adults are obese or overweight, and obesity has been associated to a heightened prevalence rate of HT, CVD, DM, and HF. Furthermore, obesity is an essential risk factor for the development of MI in people of all ages (Amen, S. O. *et al.*, 2020). Obesity in adulthood is one of the characteristics of malnutrition noticed in Iraqi citizens nowadays. Furthermore, people with cardiac problems have been found to have weight problems in some circumstances.The Table 4.4was showed the mean of salusins-α for eachgroup. The results from the analysis showed that there have been a significant ($p<0.05$) decrease of salusins-α value in BCC and in ACC patients compared to control. Additionally, the results indicated a significant ($p<0.05$) decrease in BCC patients when compared to ACC patients groups.

The result in Table 4.5 was shown the mean of salusin-β in BCC and ACC patients group were higher than the mean of control subjects (significant correlation $p< 0.05$ in these groups). Our results point out that the decrease in salusin-α levels and the rise in salusin-β levels are linked to the development of CVD. The results agree with Sato, K. *et al.* (2013), who reported that salusin plays a big role in the development of atherosclerosis. People, who have more salusin-β in their bodies, or less salusin-α in their bodies, have a higher risk of having atherosclerosis. Salusin-α and salusin-β have different effects on atherosclerosis. The ROC curve analysis indicated the good performance of salusin-α and salusin-β in diagnosis (Figure 4.1). According to a curve analysis, salusin-α levels are the best indicators for identifying people with CVD (cut-off value 95 pg/mL; AUC=0.997; 95% CI: 0.952 to 1.000). Similar findings have been published in the salusin- β cut-off point of 214 pg/mL, AUC= 0.997. 95 % CI: 0.948 to 1.000). Salusins are peptides with many functions that influence hemodynamics. They play a role in hypertension, atherogenesis, CVD, and other illnesses. Salusins are thought to play a function in the development and progression of atherosclerosis (Sipahi, S. *et al.*, 2019). According to Fujimoto, K. *et al.* (2013), salusin-β concentration levels might be a marker of peripheral artery issues. Endogenous salusin-β, on the other hand, has the potential to cause atherosclerosis. The peptides salusin- α and

salusin-β both have an influence on macrophage conversion via activating salusin- β and inhibiting salusin- α of the Acetyl-CoA Acetyltransferase 1 (ACAT-1) enzyme (Sato, K. *et al.*, 2013).

So, salusin helps to either protect and contributes to the development of atherosclerosis. Another research (Awad, A. S. *et al.*, 2020) discovered that patients with CVD had considerably greater salusin-β and significantly lower salusin-α than people without the condition. Furthermore, salusin-β rises and declines following catheterization. Table 4.6 observed that the serum salusin-α levels in human gradually decline during increasing of BMI and age in BCC and ACC patients, but not in control (may be due to the most of the patients in this group were under the age of 50 and non-obese). The results were significant ($p< 0.05$) with BMI subgroup and non-significant ($p> 0.05$) with age subgroup. Our results were shown that serum salusin-α levels are independent of age. These results agreed with Watanabe, T. *et al.* (2008), the study evaluated serum salusin-α concentrations were no significant differences in age and carotid atherosclerosis in HT patients. According to Zafrir, B. *et al.* (2018), patients who underwent cardiac catheterization had a decreased risk of long-term mortality if they were overweight or obese, but not if they were severely obese. Underweight patients were determined to be the most dangerous. The BMI increased across all age categories, but it decreased in the very old age group ($p<0.05$), and there was also a link between obesity, HT and T2DM (Canning, K. L. *et al.*, 2014).Salusin-β levels in humans increased faster in adults over 50 years old than in those under 50, and with rising BMI, as indicated in Table 4.7. The current study found that serum salusin-β levels are age-independent and BMI-dependent. These findings were consistent with those of Awad, A. S. *et al.* (2020), who examined serum salusin-β levels in CVD patients undergoing catheterization and discovered a strong association with BMI but no significant link with age. Table 4.8 shows that the irisin level in BCC and ACC patients was statistically ($p<0.0001$) lower than the mean control. There was also a significant ($p= 0.0127$) drop in serum irisin levels in BCC patients compared to the ACC group. Allehibi, K. I. *et al.* (2020) discovered similar results, demonstrating that irisin was decreased among individuals with long-standing DM (with or without CAD), which may be used as a marker for assessing the severity of DM and predicting CAD. Irisin has been implicated in a variety of CVD. Oxidative stress, which causes irisin to be produced while antioxidants prevent it, is considered to regulate irisin levels in the blood. Despite the fact that human studies have established a substantial relationship between circulating irisin levels and a

variety of CVD, this remains the case (Silvestrini, A. *et al.*, 2019). This notion is validated by our results, which reveal an inverse relationship between irisin and CVD. Patients who have a high serum irisin level within 28 days of having an AMI are also more likely to have a recurrence of the condition and to die Hsieh, I. C. *et al.* (2018). In most observational studies, patients had lower irisin levels than controls. While other research have found a link between irisin and CVD. The control of irisin in humans remains unknown, since research published to far have yielded contradictory results about the relationship between irisin levels and CVD. The statistical analysis of our findings revealed that the ROC curve analysis in Figure 4.2 suggested that irisin was a good diagnostic in CVD patients, with a cut-off value of 1.3, sensitivity of 85.71 percent and specificity of 97.4 percent, and AUC of 0.997 (95 percent CI 0.952-1.000).Table 4.9 shows the relationship between serum irisin, age and BMI in the analyzed groups. However, the influence of BMI on circulating irisin has been debated, and there is little evidence that circulating irisin is implicated in CVD in adults. Several researchers (Khorasani, Z. M. *et al.*, 2019) evaluated irisin levels, which were considerably higher in the DM patients without CAD group compared to the CAD patients ($p = 0.048$), and irisin levels exhibited a strong positive link with BMI (r= 0.374, $p = 0.004$). Furthermore, our findings show that cardiac catheterization patients in the age group 50 years had greater levels of irisin than those observed in the age group 50 years in ACC patients and the control group.. While serum irisin levels steadily fall with age (50 years) than in BCC patients. A research conducted by Fukushima, Y. *et al.* (2016) found a negative link between irisin and age and a positive correlation between irisin and percent body fat in all male individuals.

The present data in Table 4.10 was shown a significantly ($p<0.05$) higher level in the serum TC, LDL, TG, VLDL levels accompanied with a low in the HDL level in the ACC and BCC groups, compared to control group. It also found that there were significant ($p<0.05$) decreases in serum TC, LDL, TG, VLDL, and high HDL levels in the ACC group when compared to the BCC group. These findings might be attributed to obesity, as BMI is highly associated with dyslipidemia (Bdair, B. W. H. *et al.*, 2020), as well as IR (McLaughlin, T. *et al.*, 2005). Cardiovascular disease and dyslipidemia have been linked to a person's BMI. As we become older. However, the current study's findings corroborated a prior investigation that found a robust association between dyslipidemia and CVD patients with high sensitivity and specificity (McLaughlin, T. *et al.*, 2005). According to some experts, IR produces a multitude of metabolic changes that result in development of CVD.

Dyslipidemia can be caused by IR as well as insulin signaling problems, which can lead to endothelial dysfunction; all of these factors contribute to the development of atherosclerotic plaques. IR in the heart causes CVD in at least three ways: (1) interruption of signal transduction, (2) improper substrate metabolic control, and (3) a shift in myocardial substrate supply (Ormazabal, V. *et al.,* 2018). The findings are consistent with those of Awad, A. S. *et al.* (2020), who reported on the levels of lipid profile in the serum of CVD patients undergoing catheterization and comparing these levels to those of the healthy group. Inflammation and oxidative stress are both associated to atherosclerosis, which is the leading cause of most CVD. Inflammation and atherosclerosis are triggered by factors such as oxidized lipids produced by the LPO (Zhong, S. *et al.,* 2019). People with "atherogenic dyslipidemia," or a high TG level and a low HDL level, may be able to determine if they are at risk for atherosclerotic cardiovascular disease (Lee, J. S. *et al.*, 2017). LDL is a component of plaque development, but it also has a wide variety of impacts on vascular function, making atherosclerotic cardiovascular disease more likely. It's also associated with a higher risk of atherosclerotic cardiovascular disease (Zhang, Y. *et al.*, 2021). High levels of TG-rich lipoproteins in the blood increase the risk of atherosclerotic cardiovascular disease. TG does not have a direct function in the production of arterial plaque. When persons with mild to moderate hypertriglyceridemia, some of the TC that accumulates in their arteries originates from VLDL particles or their remnants, or IDLs, generally known as "bad cholesterol" (intermediate-density lipoproteins). Nonetheless, HDL levels continue to be an excellent predictor of the risk of atherosclerotic cardiovascular disease. As a result, HDL can have both positive and negative implications, and HDL may only integrate a portion of its biological tasks. While HDL levels may not be the optimum treatment goal, some have suggested that HDL function be considered instead (Hegele, R. A., and Tsimikas, S., 2019). Finally, the current study found that dyslipidemic individuals are more likely to develop CVD.

In **Table 4.11**, the mean serum glucose level before catheterization patients (BCC) and the mean of after catheterization (ACC) were statistically ($p<0.05$) higher than control group. An analysis of 20 research indicated that those without diabetes were more likely to develop CVD than people with diabetes (Coutinho, M. *et al.,* 1999). Furthermore, there was no connection (P>0.05) in blood glucose levels between the ACC and BCC groups The results may be duo to that there were few numbers of patients with high fasting serum glucose levels may be correlated with the BMI and IR which lead to increased risk of

CVD. Similar results were agreed with the study by Cast *et al.*, (2012) that was an increase in HOMA-IR was linked to more CVD than an increase in fasting glucose or fasting insulin concentration by the same amount. Including HOMA-IR in a technology that predicts CVD risk might be beneficial. Other studies have found that prolonged fasting glucose rise, even at levels lower than the diagnostic threshold for DM, is linked with an increased risk of CVD, suggesting that DM is a strong effectuated of HT. Furthermore, there is a substantial risk of developing CVD in adolescents with T2DM. As a result, using teenage pre-diabetes as a predictor of the likelihood of developing CVD in adulthood is not implausible (Bdair, B. W. H. *et al.*, 2020).

Table 4.12 was also shown that fasting serum glucose was significantly higher with higher BMI. Studies that looked at IR can induce an imbalance in glucose metabolism that generates hyperglycemia (Ormazabal, V. *et al.*, 2018). Similar study was reported by Reaven (2011) was noted that fasting glucose concentrations of all three BMI groups were different from each other. In our study, we found that glucose levels in the more than 50-year-old group were higher than they were in the less than 50-year-old group, but not any correlation that appear ($p>0.05$) between the ACC and control groups and ($p<0.05$) in ACC group only. The American Diabetes Association (2004) reported that there was a relationship between normal blood glucose and CVD risk in Asian and Australasian groups included age, and the relationships were highly comparable for both sexes as well as for different age subgroups (Asia Pacific Cohort Studies Collaboration, 2004).

The fasting serum insulin level is considered to be a reliable indicator of insulin action. An evaluation of insulin sensitivity using the HOMA-IR was a statistical manipulation of fasting blood glucose tolerance and insulin concentrations with the goal of generating a more accurate measure of insulin sensitivity. The mean and standard deviation of insulin for each of the study groups is shown in Table 4.13. In both BCC and ACC groups, insulin levels are considerably significant ($p<0.05$) greater than those in the control group. Similar results were found by Awad, A. S. *et al.* (2020), who the measure the levels of insulin in the serums of cardiovascular patients who are subject to catheterization and compared these levels with the healthy group and their correlation with salusins. Our results study agreement with the results by Gast, K. B. *et al.* (2012) that directly compared glucose, insulin, and HOMA-IR values in the strength of there was a correlation with CVD.

Insulin levels increase significantly with increased obesity and increased patients' age. The findings were shown in Table 4.14. Several clinical studies demonstrated that plasma insulin levels were often elevated in patients with clinically established CVD. Katsuki, A. *et al.* (2003) concluded that the plasma insulin levels of Japanese sick men were found to be substantially higher than those of healthy individuals and an elevation in abdomen fat is linked to a reduction in insulin responsiveness as well as an advancing rate of CVD. Insulin binding to a target cell increases the ability of this cell to absorb glucose from the blood stream; in IR, this cell's ability to absorb glucose is impaired. A healthy pancreas will overcome the IR by increasing the insulin production maintaining healthy blood glucose levels. However over time the insulin secretion cells die trying to keep up with the insulin demand, which means healthy blood glucose levels cannot longer be maintained. At this point, excess glucose will build up in the bloodstream and IR has evolved (Reaven, G. *et al.*, 2004).

One of the goals of this study was to identify who was IR and at an increased risk of CVD. If glucose is measured in mg/dL and insulin is measured in μU/L, the formula for calculating HOMA-IR is as follows: (fasting insulin x fasting glucose)/405 (Matthews, D. R. *et al.*, 1985). Mean serum concentration of HOMA-IR as shown in Table 4.15. The mean of HOMA-IR were higher ($p< 0.05$) in the study group patients (BCC and ACC) than the mean of control subjects group. The results in Table 4.15 also revealed a non-correlations ($p>0.05$) increase of HOMA-IR for BCC group in comparison to ACC group. Awad, A. S. *et al.* (2020) discovered similar results when they measured the levels of HOMA IR in the pre-catheter and post-catheter groups with CVD. Gast, K. B. *et al.* (2012) conducted a comparable meta-analysis on fasting glucose, fasting insulin, HOMA-IR, and the connection with CVD. People who did not have diabetes were more likely to develop heart disease; an increase in one standard deviation in HOMA-IR concentration raised CHD risk by 46 percent, compared to a 21 percent rise in glucose levels and a 4 percent increase in insulin concentration.A lot of metabolic changes happen during IR, which leads to CVD. It can cause an imbalance in glucose metabolism, which can lead to chronic hyperglycemia, which in turn can cause oxidative stress and inflammation, which can damage cells. IR can change the lipids' pathway are made in the body, which can lead to high TG, low HDL levels, and the formation of small dense LDL. This trio, as well as endothelial dysfunction, which can be caused by insulin signalling problems, has been a major role in development of atherosclerotic plaques (Ormazabal, V. *et al.*, 2018). The

results in Table 4.16 and Appendix 7 were shown the mean±SD of HOMA-IR levels for BCC, ACC patients and control group in relation to age and BMI. IR levels rise considerably with obesity and age groupings. These findings indicate a positive significant connection between HOMA-IR and CVD risk in all research groups when compared to BMI and age.According to current research, one-third of people with the most IR are at significantly increased risk of acquiring CVD. Most people with high IR are also overweight or obese, but this does not mean that not everyone who is overweight or obese is also IR. As a result, there are a number of IR-related problems, such as glucose intolerance, hyperinsulinemia, and dyslipidemia, among those who are simply overweight or obese and also have IR.

Only patients who are overweight or obese, but also with IR, show significant improvements in metabolic abnormalities after weight loss, indicative of a stronger therapeutic effect (Reaven, G. *et al.,* 2004).These findings support prior research by Katsuki, A. *et al.* (2003), which found that young Japanese males are more likely to be overweight or obese, with high levels of visceral fat and plasma TG associated to IR. As previously stated, these people's IR, hyperinsulinemia, and dyslipidemia may raise their risk of DM and CVD (Karelis, A. D. *et al.*, 2004).

5.1. Conclusion

1. BMI was highly associated with higher cardiovascular events, lipids dysfunction, and HOMA-IR.

2. Overweight and obesity appear to be the most important risk factors for CVD, particularly in adult male obese persons, and the CVD risk increased with rising BMI and decreased with age.

3. Salusin-α levels have a significant decrease in the serum of CVD patients and salusin-β levels have recorded a significant increase in the serum of CVD patients.

4. Salusin-α significantly decrease with increasing BMI and non-significantly with age in before and after catheterization patients. While salusin-β levels increased significantly more quickly in patients who are with increasing BMI, and increased non-significantly with increasing age before and after catheterization.

5. Our results suggest that salusin-α and salusin-β prove contrasting effects on CVD.

6. The irisin, has been considered a prognostic factor in several CVD, and our findings, confirming this suggestion, show an inverse correlation between irisin and CVD.

7. Present study showed that obesity, hyperlipidemia, diabetes and IR were the most frequent risk factors for CVD with a higher incidence in older age. Furthermore, this study gives novel data on the prevalence of CVD in the Iraqi population..

8. Assessment of salusinα, salusin-β and irisin can be used as a diagnostic tool for CVD in clinical practice and as a prognostic tool for CVD risks in Iraqi population.

5.2. Recommendations

Hyperlipidemia, obesity, and diabetes are the most frequent risk factors for CVD, and they are more common in older male people. For these reasons, our research can help with the prevention and management of certain risk factors. Obesity management and improved physical activity are the most effective approaches to minimize hyperglycemia, and reductions in IR may also reduce the risk of CVD in Iraqi populations.

To validate these findings and better understand the long-term advantages of early diagnosis and weight loss, longitudinal studies are needed. Further studies of the influence

of salusin-α, salusin-β and irisin function on the various forms of CVD in women and in patients with diabetes (T1DM and T2DM) or other metabolic diseases are still required to gain more insight into the relationship between parameters and CVD.

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Appendixes

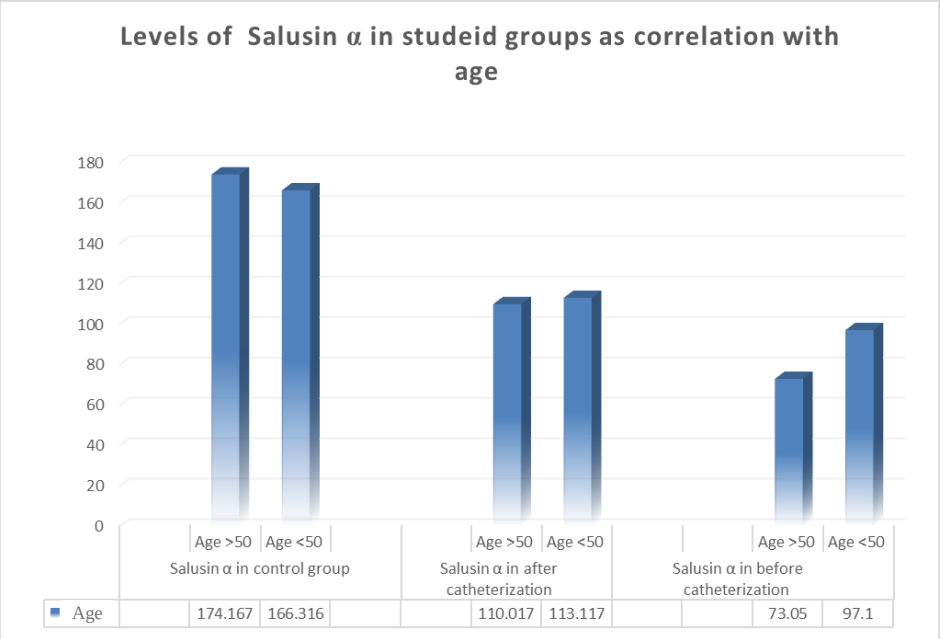
Appendix 1: Questionnaire

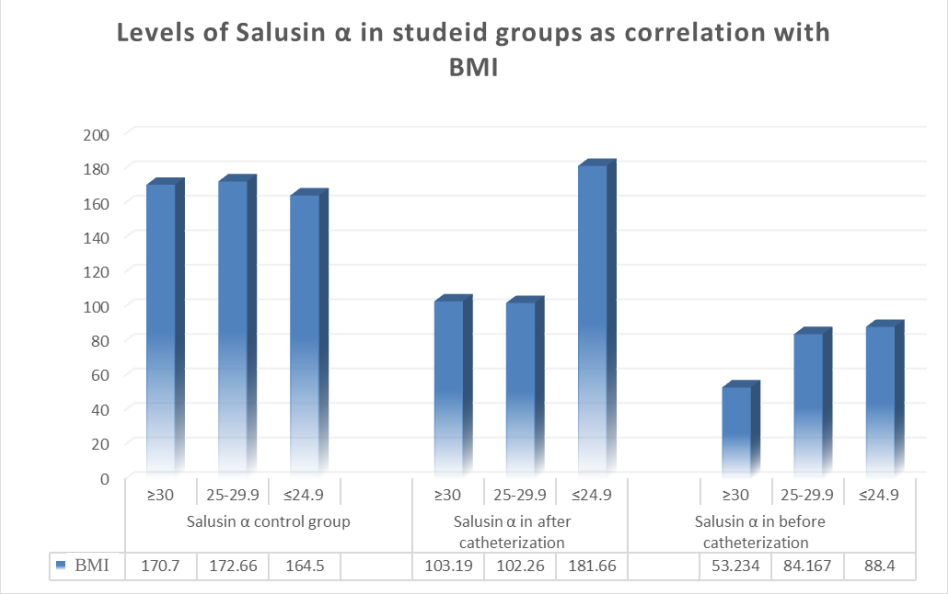
Cardio Vascular Disease Questionnaire

1) **Name** …….. …… …….. ……
2) **Mobile No**……..
3) **Weight** □□**Height** □□ **BMI** □
4) **Where haveyou lived?** ……
5) **How old are you?** □□years
6) **When were you born?** Day Month Year
□□□□□
7) .**Are you fasting?** Yes □ No□
8) **History of Cardiovascular disease:**
Atherosclerosis □
Cardiomyopathy □□□
Congenital heart disease □□
Heard failure □□□
Heart valve disease □□□
Other: specify……..□
9) **Have you had a cardiac catheterization?** Yes □ No□
If yes, please indicate when? Day Month Year
□□□□□
10) **Do you have an appointment for a cardiac catheterization procedure?**
Yes □ No□
If yes, please indicate when? Day Month Year
□□□□□
11) **Do you have diagnosed with any of the following?**
- Diabetes Yes □ No□
- Smoking history Yes □ No□
- Family History of CVD Yes □ No□
- Blood pressure Yes □ No□
- Kidney disease Yes □ No□
- Liver disease Yes □ No□
- Thyroid disease Yes □ No□
- Cancer Yes □ No□
-Others ……

Appendix 2:

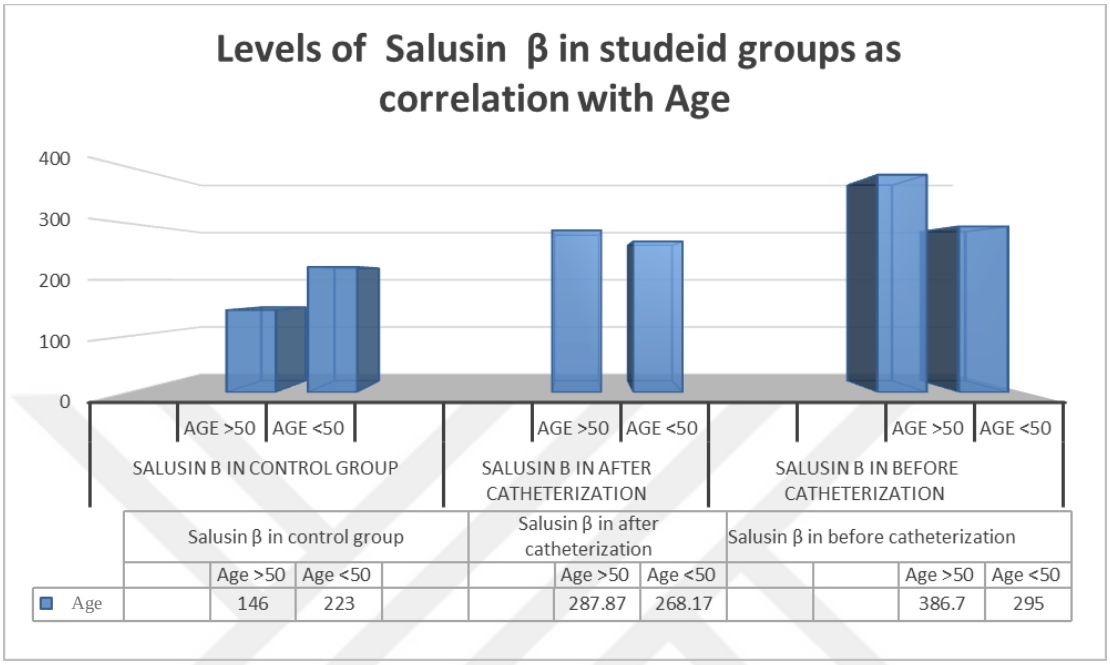
The levels of Salusin-α for BCC, ACC patients and control group as correlation with age and BMI



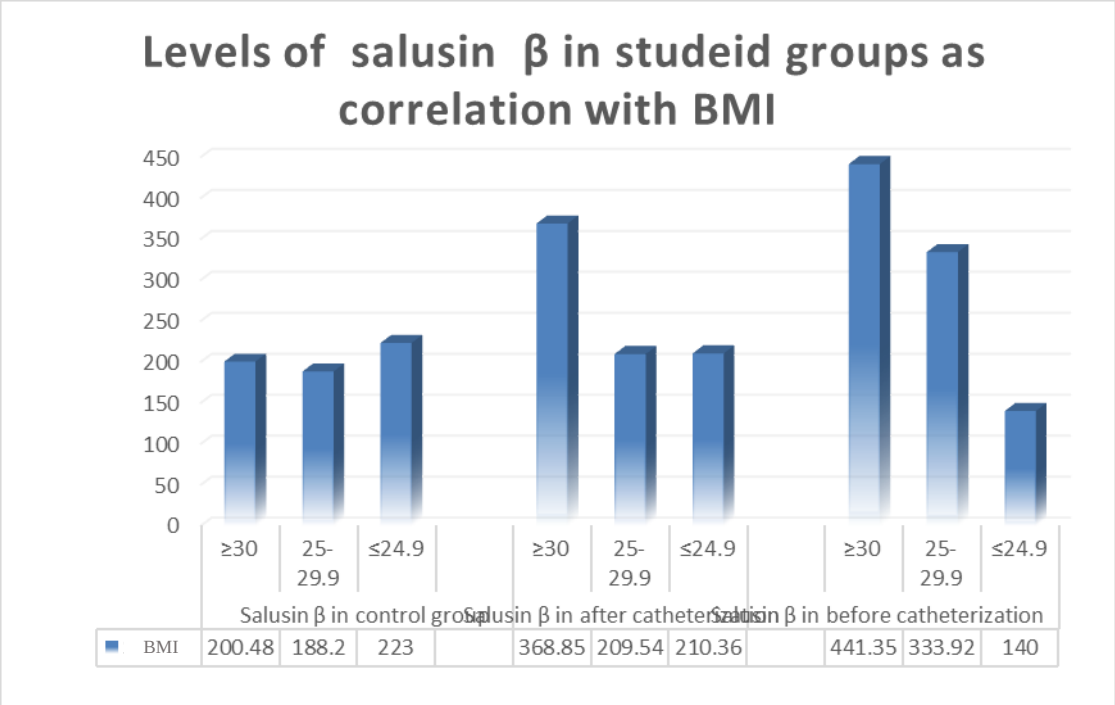


Appendix 3:

The levels of Salusin-β for BCC, ACC patients and control group as correlation with age and BMI



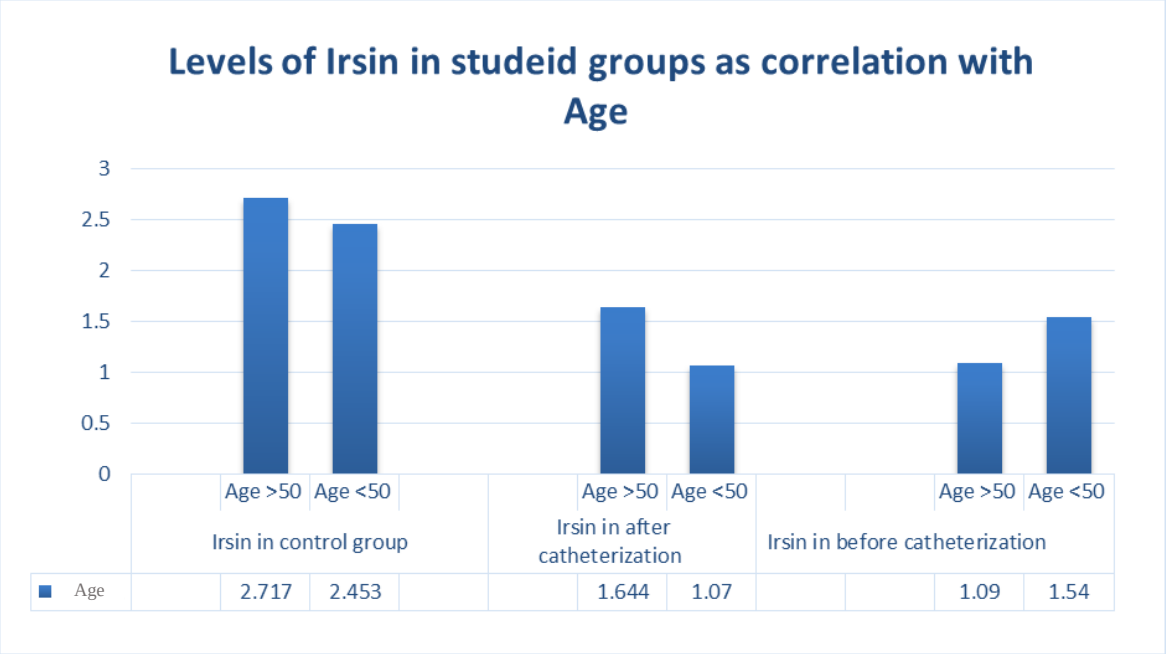
	Salusin β in control group			Salusin β in after catheterization			Salusin β in before catheterization			
		Age >50	Age <50			Age >50	Age <50			Age >50	Age <50
Age		146	223			287.87	268.17			386.7	295

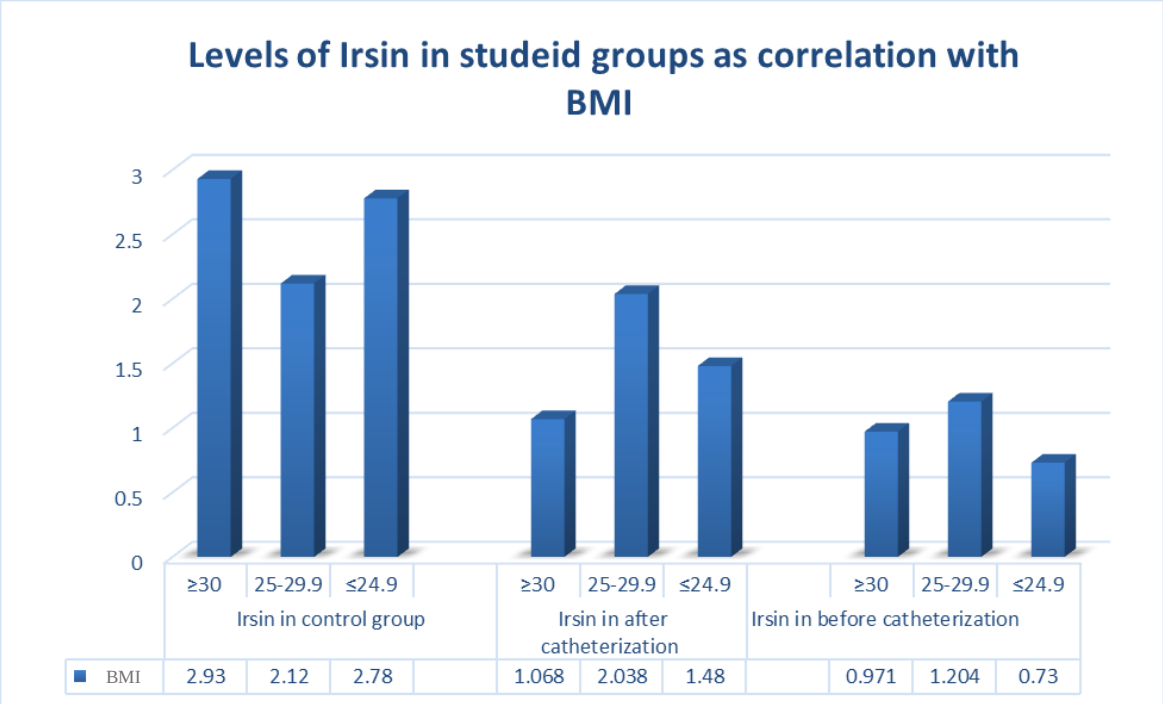


BMI	200.48	188.2	223		368.85	209.54	210.36		441.35	333.92	140

Appendix 4:

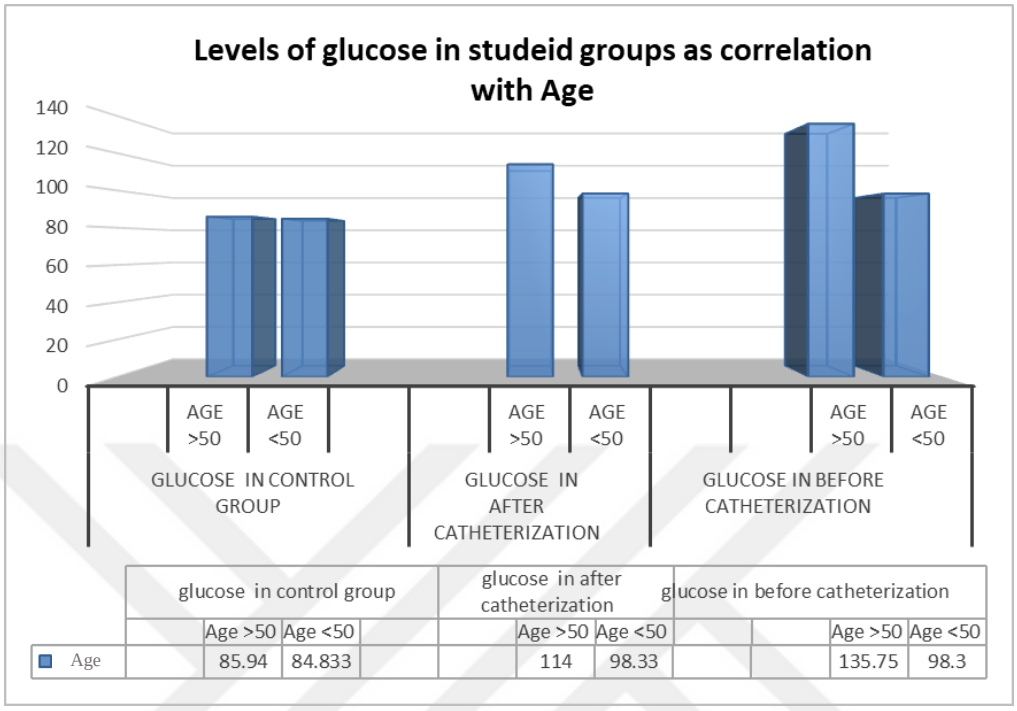
The levels of Irisin for BCC, ACC patients and control group as correlation with age and BMI



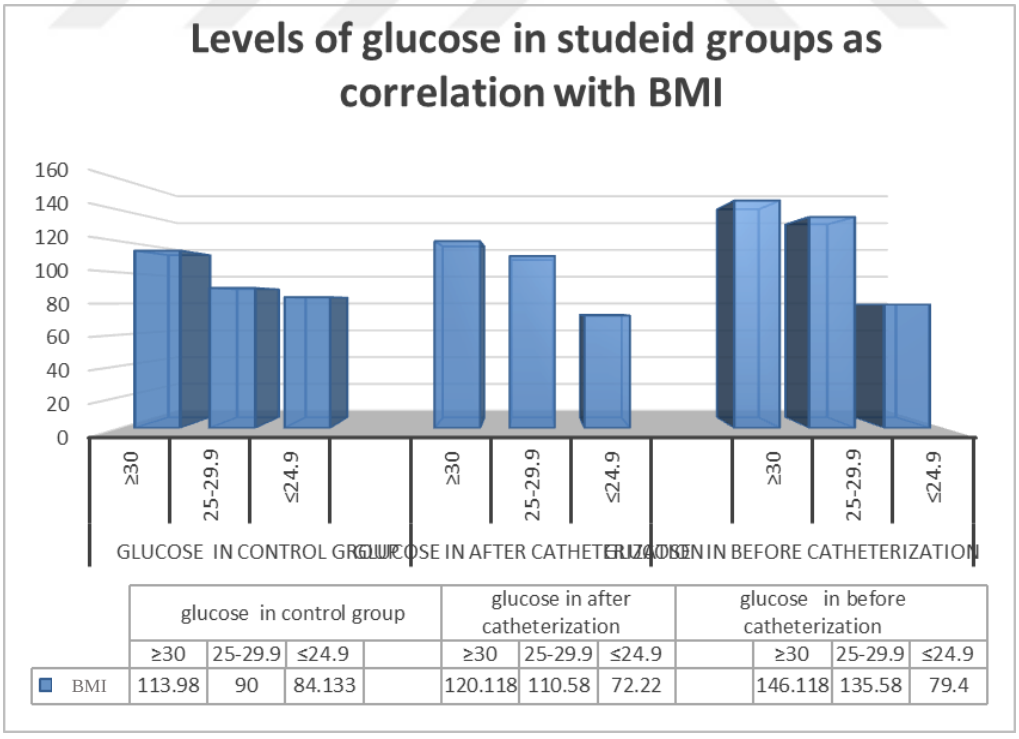


Appendix 5:

The levels of glucose for BCC, ACC patients and control group as correlation with age and BMI



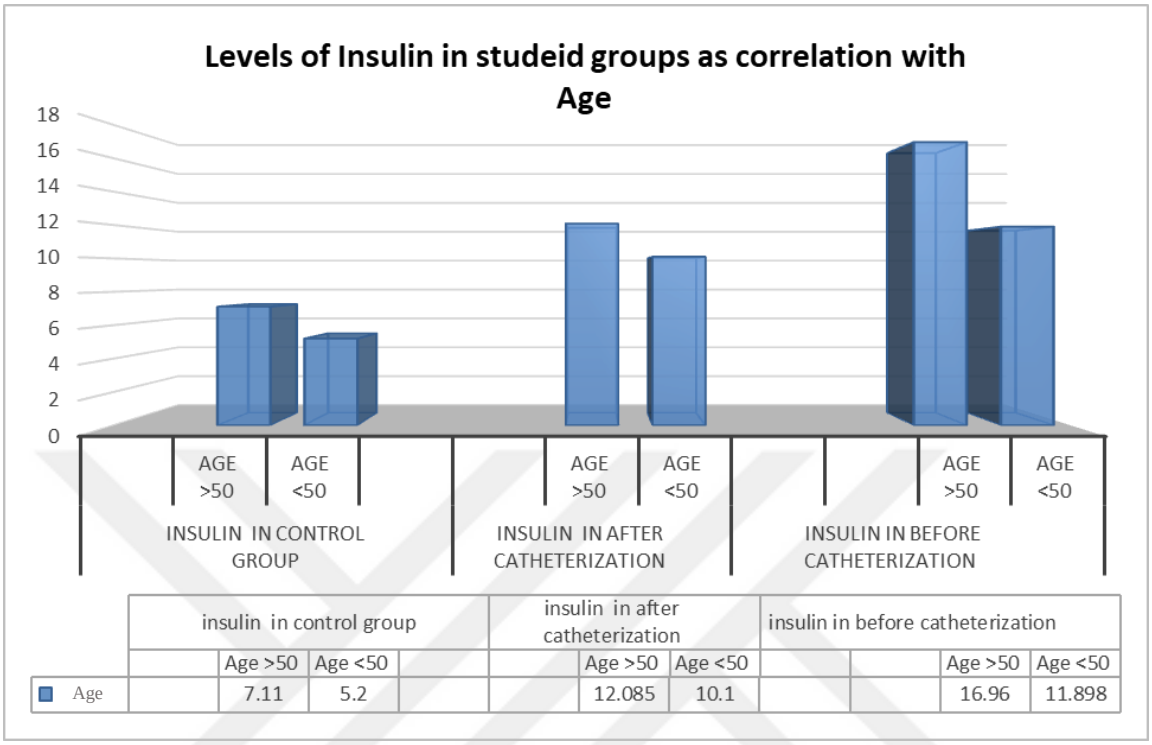
	glucose in control group			glucose in after catheterization			glucose in before catheterization		
	Age >50	Age <50		Age >50	Age <50		Age >50	Age <50	
Age	85.94	84.833		114	98.33		135.75	98.3	



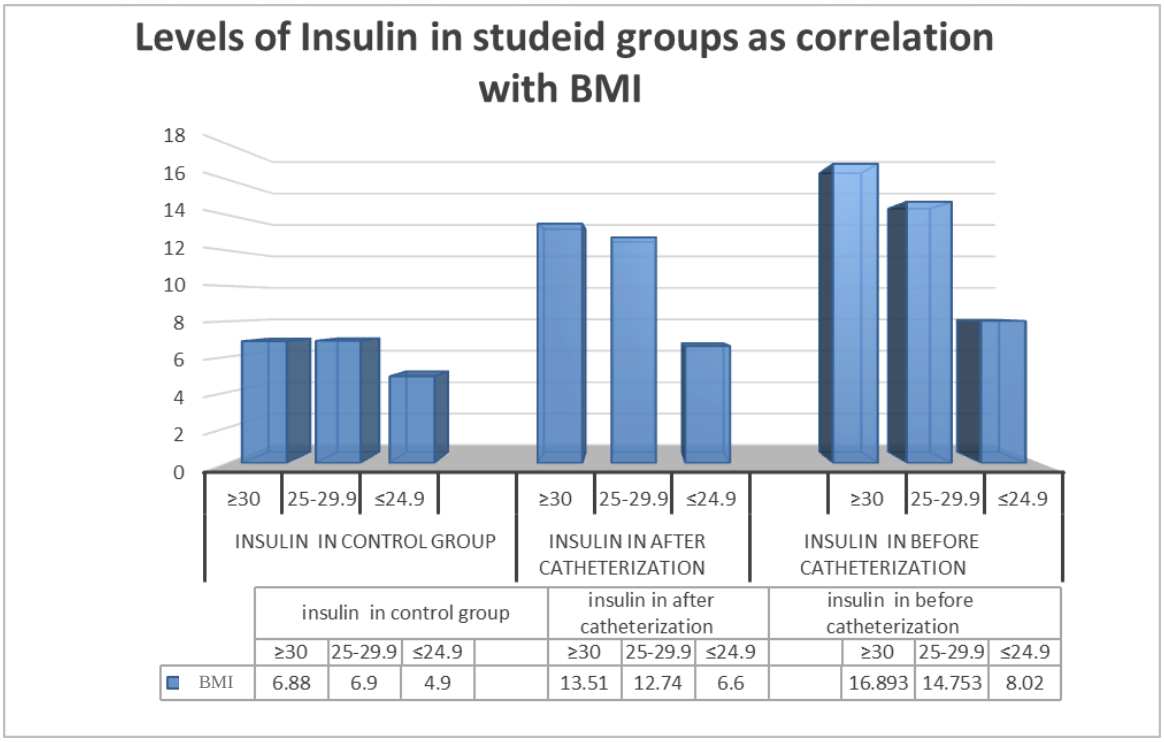
	glucose in control group			glucose in after catheterization			glucose in before catheterization		
	≥30	25-29.9	≤24.9	≥30	25-29.9	≤24.9	≥30	25-29.9	≤24.9
BMI	113.98	90	84.133	120.118	110.58	72.22	146.118	135.58	79.4

Appendix 6:

The levels of Insulin for BCC, ACC patients and control group as correlation with age and BMI



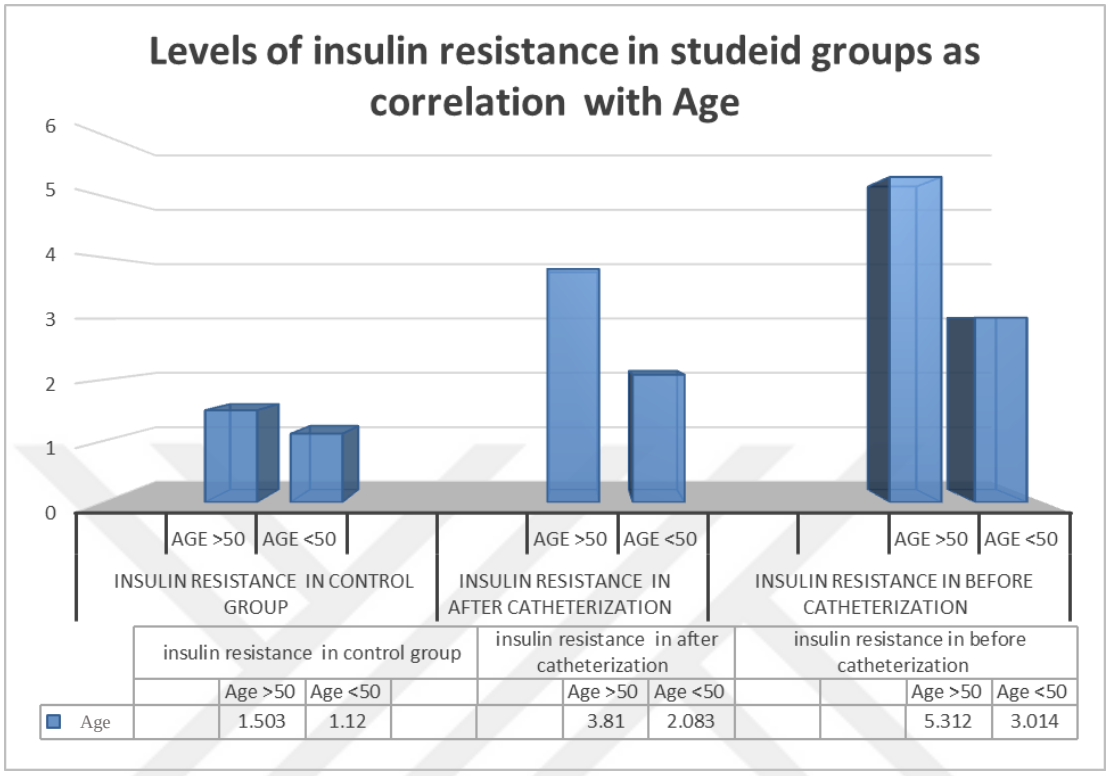
	insulin in control group			insulin in after catheterization			insulin in before catheterization			
		Age >50	Age <50			Age >50	Age <50			Age >50	Age <50
Age		7.11	5.2			12.085	10.1			16.96	11.898



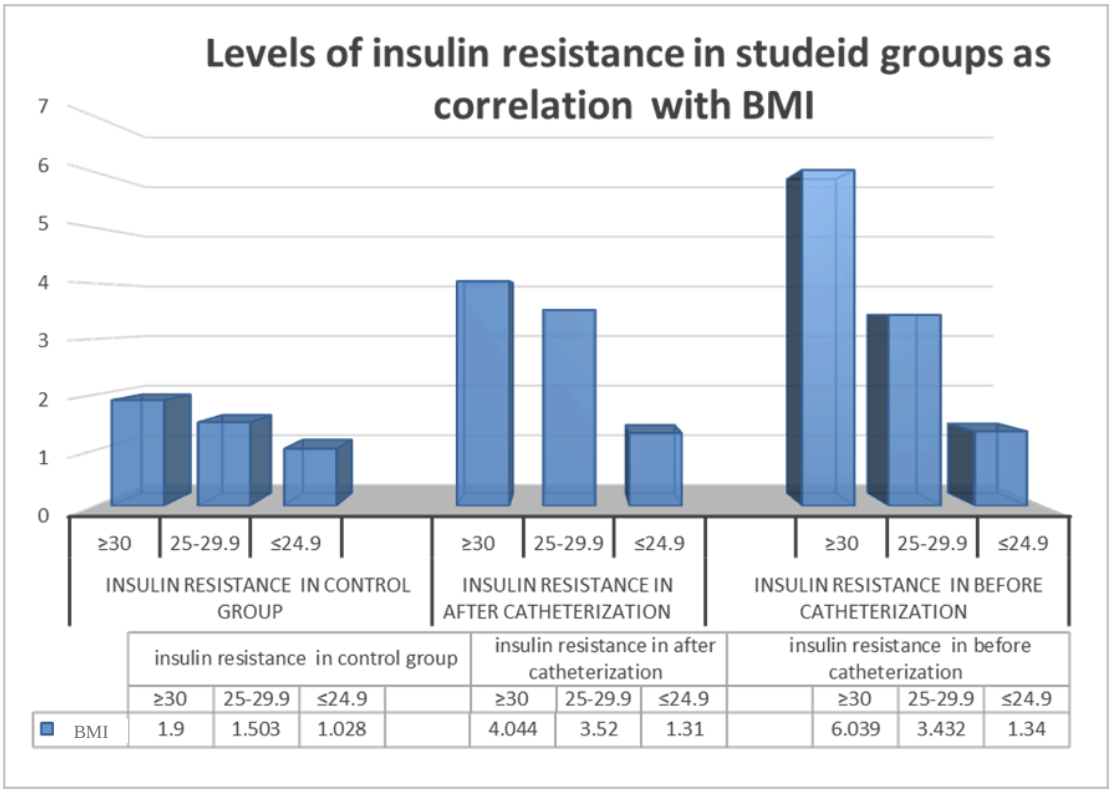
	insulin in control group				insulin in after catheterization				insulin in before catheterization			
	≥30	25-29.9	≤24.9		≥30	25-29.9	≤24.9		≥30	25-29.9	≤24.9	
BMI	6.88	6.9	4.9		13.51	12.74	6.6		16.893	14.753	8.02	

Appendix 7:

The levels of Insulin resistance for BCC, ACC patients and control group as correlation with age and BMI



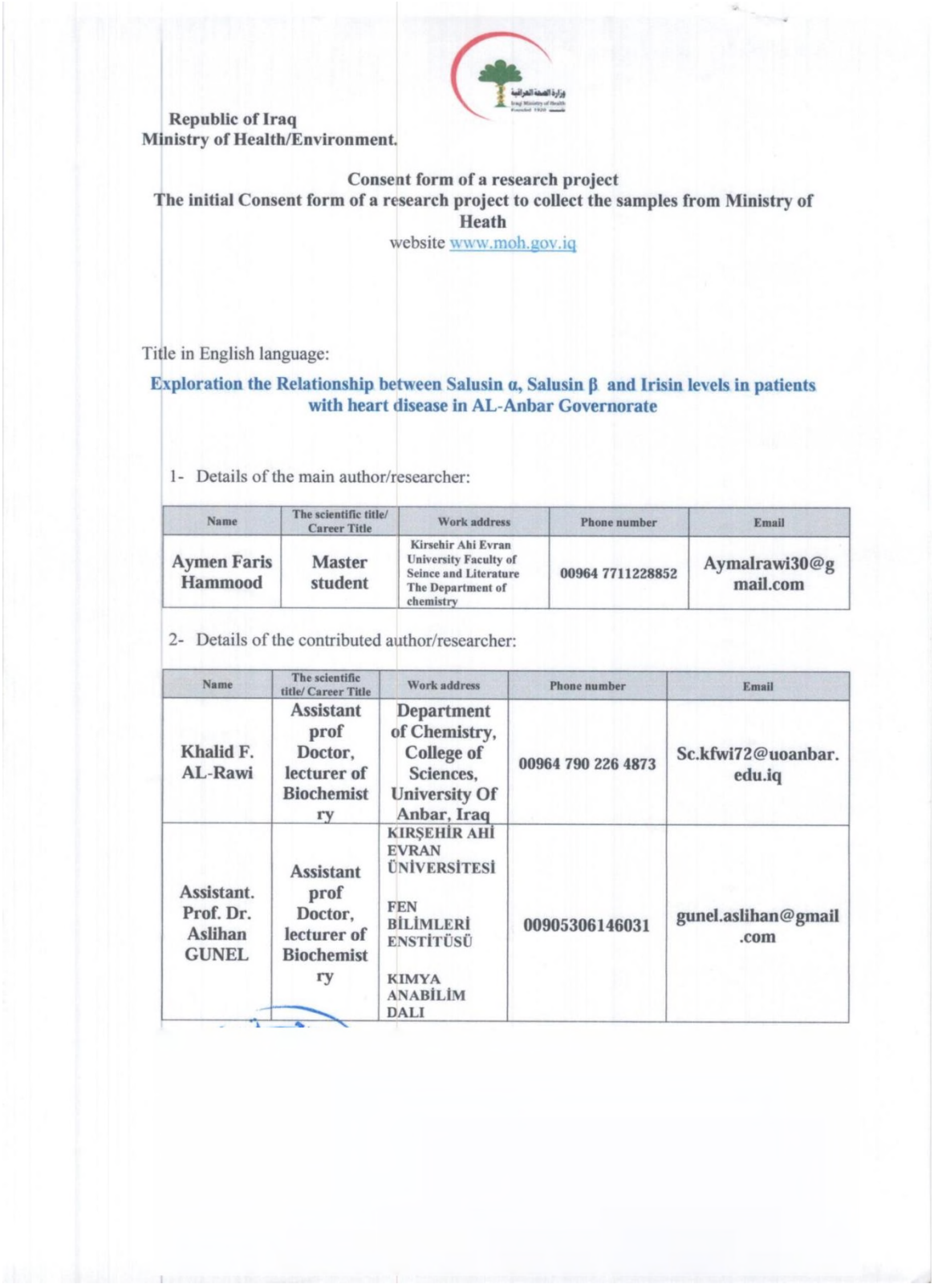
	insulin resistance in control group			insulin resistance in after catheterization			insulin resistance in before catheterization			
		Age >50	Age <50			Age >50	Age <50			Age >50	Age <50
Age		1.503	1.12			3.81	2.083			5.312	3.014



	insulin resistance in control group			insulin resistance in after catheterization			insulin resistance in before catheterization			
	≥30	25-29.9	≤24.9	≥30	25-29.9	≤24.9	≥30	25-29.9	≤24.9	
BMI	1.9	1.503	1.028	4.044	3.52	1.31	6.039	3.432	1.34	

Appendix 8:

Permission to collection of samples to complete a master's thesis



Republic of Iraq
Ministry of Health/Environment.

Consent form of a research project
The initial Consent form of a research project to collect the samples from Ministry of Heath
website www.moh.gov.iq

Title in English language:

Exploration the Relationship between Salusin α, Salusin β and Irisin levels in patients with heart disease in AL-Anbar Governorate

1- Details of the main author/researcher:

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3- The Scientific Supervisor/ if found

Name	The scientific title/ Career Title	Work address	Phone number	Email
None				

A. Work background:

Find the correlation between Irisin, salusin, lipid content, blood sugar level, and insulin resistance.

Compare levels of new evidence by age, gender, and more

B. The importance of the research and its objectives:

The aim of this study is to explore the association between various biochemical and immunological parameters of male patients with Measuring the level of lipids, blood sugar, insulin, insulin resistance, irisin, salosin, and in the serum of heart patients

Those undergoing catheterization and compared with the healthy group.and also to perform a predictive equation linking these parameters together.

C. Conclusion:

The study revealed some factors that would have a positive or negative effect on patients with heart disease .

4- People or martials that are required for this research from the Ministry of Health.?

Martials	Type
Laboratories samples (blood, stool, urine and Smears from the site of injury)	Yes, blood samples, patients and controls length, twits, weight and Blood pressure measurement
Martials/ equipment's	No need (Privet)
information from the patients Records	No need
Patients or other staff members	The study included a total of 60 male patients suffering from heart disease. With 25 health controls
Others	No

5- Time and the date to perform the research: (suggested locations)

Time: 1st April 2021- 1st Jun 2022.
Locations:

Name of institute	Approval
Private and Governmental laboratories in Ramadi and Falluja cities	

6- Fund: None.

7- Methodology:

A. Study design: Blood samples and information were collected from 60 cardiac patients with 25 healthy controls
B. Case definition and exclusion criteria for negative tests and sampling methods were considered..
C. Physiological and biochemical Parameters were measured.
D. The expected number of sampling: 100, 50 patients and 50 controls
E. Statistical analysis: statistical descriptive tables and maths correlations.
F. Ethical consideration during research: patients names must not menti

G. Signed Commitment:

This is **AYMEN FARIS HAMMOOD** I signed below to commit that I perform the research according to this protocol. Also, I commit that I will never change or modify it after it is being approved unless agreed with research committee in the health institute. Moreover, I commit following the laws, rules and instructions of Iraqi health ministry and any other official parties that follow the scientific and ethical commitment for research.

Name and the signature of the main researcher: **Aymen Faris Hammood**

The name and the signature of the supervisor/ if the research is performed to obtain a BSc, MSc or PhD etc: None.

Approval of the research committee at the health institution (or the body authorized to approve this form)

CURRICULUM VITAE

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Date of birth	
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