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EVALUATION OF SERUM OSTEOPONTIN (OPN) LEVELS IN PATIENTS WITH ISCHEMIC HEART DISEASE

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
CHEMISTRY

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MOHAMMED ABDULLAH HASSAN HASSAN

ÇANKIRI

2022

EVALUATION OF SERUM OSTEOPONTIN (OPN) LEVELS IN PATIENTS WITH ISCHEMIC HEART DISEASE

By Mohammed Abdullah Hassan HASSAN

Aralık 2022

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

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ABSTRACT

EVALUATION OF SERUM OSTEOPONTIN (OPN) LEVELS IN PATIENTS WITH ISCHEMIC HEART DISEASE

Mohammed Abdullah Hassan HASSAN

Master of Science in Chemıstry

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December 2022

Ischemic heart disease affects the heart as a result of narrowing of the arteries, resulting in less oxygen and blood reaching the heart muscle. The reason for using age in this study is that there is a strong link between age and IHD, which is that the older a person grows, the higher their risk of developing IHD. Since diabetes and cardiovascular disease are close associated, and cardiovascular disease is the leading cause of death, glucose and HbA1C were measured in this study. An increase in the percentage of fats above the normal level (cholesterol, LDL, VLDL and triglycerides), and any abnormal increase in these percentages leads to a higher chance of getting IHD, and there is a role of HDL (which is less than the normal limit in these cases) in this morbidity. May lead to more serious health problems. Excess fat in the circulatory system leads to blockage of blood vessels, especially in the coronary arteries, high blood pressure, and ischemic heart disease, leading to a heart attack. The serum level of hs-CRP was measured in people with IHD by ELISA. High serum levels of hs-CRP correlate with the risk of IHD. We found a strong link between Osteopontin (OPN) and biochemical factors. In addition, OPN had a positive association with age, height, BMI, and FBS. OPN, FBS, BMI, and HbA1c all show a positive relationship.

2022, 46 pages

Keywords: Osteopontin, Ischemic heart disease, Lipid profile.

ÖZET

İSKEMİ KALP HASTALIĞI OLAN HASTALARDA SERUM OSTEOPONTİN (OPN) DÜZEYLERİNİN DEĞERLENDİRİLMESİ

Mohammed Abdullah Hassan HASSAN

Kimya, Yüksek Lisans

Tez Danışmanı: Doç. Dr. Şevki ADEM

Eş Danışman: Dr. Öğr. Üyesi Ammar Lateef HUSSAİN

Aralık 2021

İskemik kalp hastalığı, kalbin atardamarlarının daralması nedeniyle kalbi etkileyen, atardamarların daralmasına yol açan ve kalp kasına kan ve oksijen iletiminin azalmasına neden olan bir hastalıktır. Bu çalışmada yaş kullanılmıştır çünkü ilerleyen yaş ile İKH arasında açık bir ilişki vardır, yani yaş ne kadar yüksekse İKH riski de o kadar fazladır. Ayrıca diyabet ve en yaygın ölüm nedeni olan kardiyovasküler hastalık arasında yakın bir ilişki vardır, bu nedenle bu çalışmada glukoz ve HbA1C test edilmiştir. Normal seviyenin (kolesterol, LDL, VLDL ve trigliseritler) üzerindeki yağ yüzdesindeki artış ve bu yüzdelerdeki herhangi bir anormal artış, İKH gelişme riskinin artmasına neden olur ve HDL'nin (normalden daha az olan) bir rolü vardır. bu vakalarda limit) bu morbiditede . Daha ciddi sağlık sorunlarına yol açabilir. Dolaşım sistemindeki fazla yağ, özellikle koroner arterlerdeki kan damarlarının tıkanmasına, yüksek tansiyona ve iskemik kalp hastalığına yol açarak kalp krizine yol açar. IHD'li kişilerde hs-CRP'nin serum seviyesi ELISA ile ölçüldü. Yüksek serum hs-CRP seviyeleri, İKH riski ile ilişkilidir. Osteopontin (OPN) ile biyokimyasal değişkenler arasında bir ilişki bulduk. Ayrıca Osteopontin (OPN) ile yaş, boy, VKİ ve FBS arasında pozitif bir ilişki vardı. Osteopontin (OPN), FBS, BMI ve HbA1c arasında da pozitif bir ilişki vardır.

2022, 46 sayfa

Anahtar Kelimeler: Osteopontin (OPN), İskemik kalp hastalığı, Lipid profili.

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Advisor Dr. Doç. Şevki ADEM , please accept my heartfelt gratitude, that allowed me to continue my research project and helped me take the right and successful path.

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I extend my great thanks to my family who stood with me throughout my study period, especially my mother and father and my wife om adam, who have always been the great foundation and moral motivator during my academic career.

And a big thank you to all my friends who encouraged me and stood with me and thanks to them I overcame the most difficult circumstances.

Mohammed Abdullah Hassan HASSAN
Çankırı-2022

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LIST OF SYMBOLS

αVβ1	Integrin alphaV beta1
αVβ3	Integrin alphaV beta3
αVβ5	Integrin alphaV beta5
α5β1	Integrin alpha5 beta1
α1β1	Integrin alpha1 beta1
α9β1	Integrin alpha9 beta1

LIST OF ABBREVIATIONS

ACS	Acute coronary syndrome
AMI	Acute myocardial ınfarction
CAD	Coronary artery disease
CCSA	Canadian cardiovascular society angina
CHD	Coronary heart disease
CIHD	Chronic ischemic heart disease
ECG	Electrocardiography
HbA1C	Haemoglobin a1c, glycated haemoglobin or glycosylated haemoglobin
HDL	High density lipoprotein
hs-CRP	High sensitive c-reactive protein
IHD	Ischemic heart disease
LDL	Low density lipoprotein
MRI	Magnetic resonance imaging
NT-proBNP	N-terminal pro b-type natriuretic peptide
ONP	Osteoponti
PVA	Prinzmetal's variant angina
TC	Total cholesterol
TG	Triglycerides
VLDL	Very low density lipoprotein

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1. INTRODUCTION

Ischemic heart disease (IHD) is a kind of cardiovascular illness that is also known as coronary artery disease (CAD). Stable angina, unstable angina, myocardial infarction, and sudden cardiac death are all included in this category. It is the most common type of cardiovascular disease in the group. IHD occurs when blood flow to the heart is restricted, causing less oxygen to reach the heart muscle. Partial or total blockage of the heart's arteries is the most common cause of reduced blood flow in people with coronary artery disease (CAD). IHD also weakens the ability of the heart muscle to pump blood in a healthy and necessary manner. A heart attack occurs as a result of a sudden blockage in one of the heart's arteries. Myocardial ischemia can also cause dangerously irregular heartbeats. A common symptom is chest pain or discomfort that spreads to the shoulder, arm, back, neck, or jaw. The patient may often feel sad. Symptoms are generally brought on by physical activity or mental stress, and they last only a few minutes before disappearing with rest. Shortness of breath is common, but generally goes unnoticed. The first symptom of cardiac disease is generally a heart attack. Other issues include heart failure and arrhythmias (Sakboonyarat and Rangsin 2018).

Risk factors include high blood pressure, smoking, diabetes mellitus, inactivity, obesity, high cholesterol, poor diet, depression, and excessive alcohol intake. The basic mechanism of atherosclerosis in the heart is a reduction in blood and oxygen flow to the heart muscle. To aid in the diagnosis, an electrocardiogram, heart stress test, coronary CT angiography, coronary angiography, and other tests may be employed (Castelli 1996).

Improving blood flow to the heart muscle is a key component of IHD treatment. The illness can be treated with medications, angioplasty (a technique to open clogged arteries), or surgery (Ho *et al*. 2006).

Osteopontin (OPN) is a T-lymphocyte activator and secreted phosphoprotein 1 that is also known as sialoprotein 1 (BSP-1 or BNSP). The SPP1 gene codes for a human

protein called SPP1. OPN is a 314-amino-acid glycoprotein that is extensively phosphorylated, has acidic characteristics, and contains a lot of aspartic acid (Nora *et al*. 1980).

An increase in the amount of lipids in the blood, such as cholesterol, LDL, and triglycerides, over the normal rate causes several serious repercussions, such as heart attacks, angina pectoris, and stroke. And don't forget the role of HDL (which is less than the normal limit in these cases), In addition, ignoring the excessive fat percentage may result in the onset of more serious health problems. Excess fat in the circulation causes blood vessel obstruction, particularly in the coronary arteries, elevated blood pressure, and ischemic heart disease, which leads to a heart attack (Rasmussen *et al*. 1989).

Therapeutic and dietary measures to reduce hyperlipidemia are sufficient to avoid developing ischemic heart disease (Varbo *et al*. 2014).

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The aim of this research is to determine the changes in serum levels of osteopontin (OPN) and LIPED PRO in patients with ischemic heart disease.

2. LITERATURE REVIEW

2.1 Ischemic Heart Disease (IHD)

2.1.1 Define of IHD

The lack of blood and oxygen to the heart is known as ischemic heart disease. Ischemic heart disease (IHD) is a kind of heart disease in which the arteries leading to the heart become narrowed. Other words for the same condition are coronary heart disease (CHD) and coronary artery disease (CAD). When the arteries constrict, less blood and oxygen reach the heart muscle, In the long run, this could result in a heart attack (Varbo *et al*. 2014).

Ischemic heart disease affects a high percentage of the population in both developed and developing countries. It's most commonly manifests itself in men as a myocardial infarction (heart attack), whereas it manifests itself in women as angina pectoris (Figure 2.1).

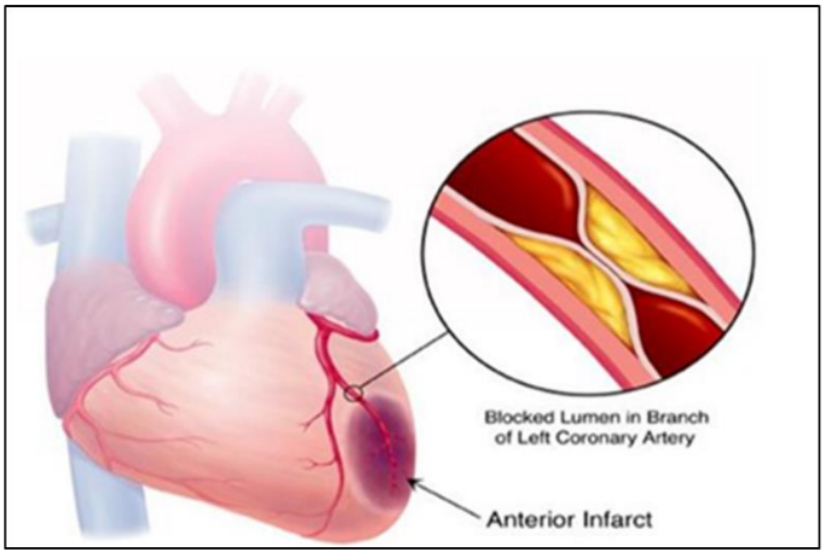

Figure 2.1 Heart diseases (Manson *et al*. 2003)

2.1.2 Type of IHD

Acute coronary syndrome (ACS): Chest pain is sometimes known as "Angina pectoris" (classical, stable angina). Even if the ischemia isn't severe enough to cause infection, the symptoms may suggest that myocardial infarction is imminent. In persons with stable angina, episodes of chest pain are common. It's a common and manageable form of anxiety. When the patient is racing or under stress, he or she is likely to come across it. The most typical treatments for chest discomfort are rest, nitroglycerin, or a combination of the two. Nitroglycerin relaxes the coronary arteries and other blood vessels, lowering the volume of blood returning to the heart and thereby lowering the workload of the heart. It improves blood flow to the heart by relaxing the coronary arteries. Thoracic aortic calcification is frequent in people who have stable angina. Calcification of the coronary arteries and valves occurs as a result of this, and it is linked to age. Thoracic aortic calcification, particularly descending aortic calcification, has been related to an increased risk of death and cardiovascular disease (Eisen *et al*. 2008).

Prinzmetal variant angina; Prinzmetal's angina is an uncommon type of angina that affects roughly 2% every people infected of angina in Figure 2.2; it affects people who are younger than those who have other types of anginas. Variant (Prinzmetal) Angina has several causes. A spasm in the coronary arteries causes the pain of variant angina (which supply blood to the heart muscle). Recurrent episodes of chest pain (angina) that frequently occur at night, between the hours of midnight and early dawn, are known as Prinzmetal's variant angina (PVA). Physical effort or emotional stress, on the other hand, are common causes of "typical" angina (Mayer and Hillis 1998).

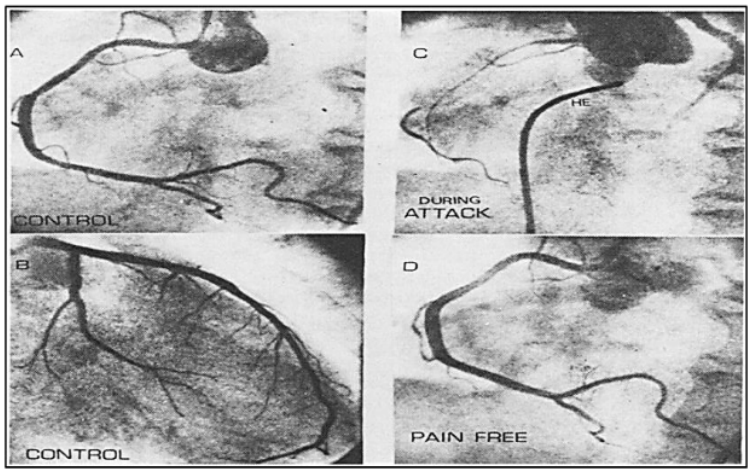

Figure 2.2 Prinzmetal variant angina (Endo *et al*. 1976)

Unstable angina: As a result of a partial blockage of a cardiac artery, unstable angina, a kind of ACS, causes sporadic or unpredictable chest discomfort. In contrast to stable angina, unstable angina pain or discomfort often occurs when resting, lasts longer, is unaffected by medication, and is unconnected to any clear reason, such as physical effort or emotional stress. Instable angina is caused by a mismatch between myocardial oxygen supply and demand. Reduced cardiac perfusion produced by a nonocclusive thrombus on a fissured or degraded atherosclerotic plaque that had previously only caused mild to moderate blockage is the most common cause. In individuals with unstable angina, coronary angioscopy and arteriography have found nonocclusive thrombi. They're particularly common on lesions that are complex and irregular. Cholesteryl esters and tissue factor are commonly found in the core of damaged plaques. Shear stresses at the plaque's shoulder disrupt a thin fibrous cover. People with unstable angina have platelet products discharged into their coronary circulation, and thrombus development appears to last for months after the initial incident. As the plaque expands, nonocclusive coronary thrombi frequently organize and become incorporated into it (Thygesen and Alpert 2001).

Myocardial infarction: is one of the leading causes of death and disability in the United States. Coronary artery disease is a long-term condition that goes through stable and

unstable periods. During times of instability, when the vascular wall is actively inflammatory, patients may have a myocardial infarction. Myocardial infarction can be a small complication of a long-term chronic condition that passes undiagnosed, or it can be a major occurrence that results in mortality or considerable hemodynamic impairment (Figure 2.3). Myocardial infarction can be the first symptom of coronary artery disease or it can occur more frequently in people who already have it (Thygesen *et al*. 2007).

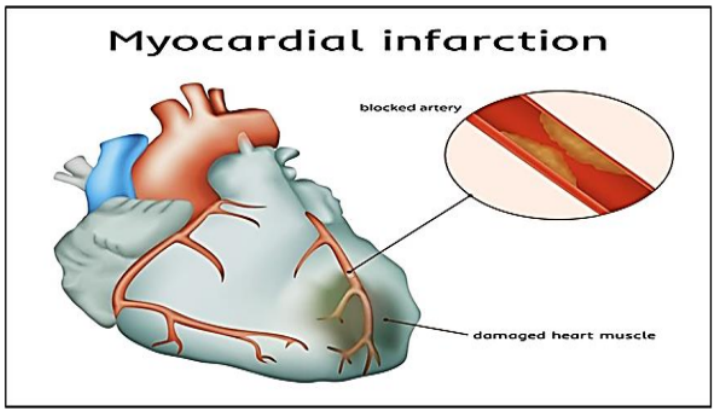

Figure 2.3 Mycardial infarction (Warriner and Al-Matok 2019)

Sudden cardiac death happens when a person dies abruptly from a cardiac cause, usually less than an hour after symptoms develop, and without any pre-existing potentially lethal ailment. A heart arrhythmia is frequently blamed for such a quick death (Connolly 2000). The rate of sudden cardiac death is approximately 50%, and it decreases with age. A variety of variables, many of which are age-related, can cause sudden cardiac death. Cardiac abnormalities, hypertrophic cardiomyopathy, and myocarditis are common substrates for fatal arrhythmias in children and adolescents. The most common findings in autopsies of people who died of sudden cardiac death are coronary atherosclerosis and acquired cardiomyopathies (Virmani *et al*. 2001).

Chronic ischemic heart disease (CIHD): is characterized by a transient mismatch that causes reversible myocardial ischemia and is characterised by constant symptoms across months, years, or even decades (Pepine and Nichols 2007).

2.1.3 Classification of ischemic heart disease

This classification is based on the Canadian cardiovascular society angina classification (CCSA) (Kaul *et al*. 2009).

- Class I: With normal activity, there is no angina. Angina exacerbated by vigorous exercise.
- Class II: Angina during everyday activities, such as going up hills or quickly ascending stairs, with a moderate restriction of activities.
- Class III: Low-intensity exercise, such as walking 50–100 yards on flat ground or climbing one flight of stairs, and a considerable restriction of activities are associated with angina.
- Class IV: Angina can strike at any time and with any level of exercise.

2.1.4 Cause of ischemic heart disease

Coronary artery disease is thought to begin when a coronary artery's innermost layer is damaged or harmed, which can occur as early as childhood. Ischemic heart disease occurs when the lumen of the coronary arteries narrows, which can happen for a variety of causes. As a result, the heart muscle's blood and oxygen supply is reduced. The reasons for the narrowing of the vessel's diameter can vary. Atherosclerosis is the most common cause. Other causes of IHD are:

- Smoking
- High blood pressure
- High cholesterol
- Diabetes mellitus

- Lack of exercise (sedentary lifestyle)
- Spasm of blood vessels (unstable angina)
- Obstruction (a foreign body entering the coronary arteries)
- Thrombosis (The most common cause of thrombosis in the coronary arteries is atherosclerosis).
- Tumor, an aortic aneurysm, or another tumor-like condition (They have the ability to exert external pressure on the coronary arteries).

When the inner wall of the artery is wounded, cholesterol and other cellular waste products tend to develop fatty deposits (plaques) near the site of injury. The medical word for this ailment is atherosclerosis. Platelets clump together at the location of the plaque rupture or fracture in an attempt to repair the artery. This clot has the potential to obstruct a blood vessel, resulting in a heart attack (Ahmed and Creager 2017).

2.1.5 Risk factors for IHD

IHD has a number of risk factors.:

- Smoking; Smokers have a seven-fold heart disease risk is higher (Yarnell *et al*. 1991).
- High Blood Pressure; If a person has high blood pressure, the risk of a heart attack or stroke doubles. Women over the age of 65 are more sensitive to high blood pressure, with 40% of them suffering from it (Abbas *et al*. 2009).
- High Cholesterol; High levels of harmful LDL cholesterol (as opposed to "good" HDL cholesterol) lead to heart disease by narrowing coronary arteries (Lamarche *et al*. 1996).
- Diabetes; People with diabetes are more likely to have other heart disease risk factors such as high blood pressure, high cholesterol, and obesity (Sakboonyarat and Rangsin 2018).

- Physical Inactivity; Overweight people are more prone to suffer a heart attack, particularly if the weight is concentrated around the waist and upper torso (compared to extra weight around the hips) (Lippi and Sanchis-Gomar 2020).
- Psychosocial Issues; Higher rates of cardiovascular disease are linked to stress, depression, social isolation, and a lack of social support. Stress has an effect on the body, making the heart work harder and perhaps worsening high blood pressure. Depression can have an impact on a person's rehabilitation and how well they recover from a heart attack (Roncella 2019).
- Family History; A family history of IHD is an independent risk factor for acute myocardial infarction (AMI), therefore addressing modifiable risk factors may be beneficial even for those with a family history of the condition. There are numerous dimensions to the link between IHD in the family and the risk of AMI (Bertuzzi *et al*. 2003).
- Menopause; A woman's estrogen level drops as she approaches menopause, but her blood pressure, cholesterol, and other blood fat levels rise. A woman's risk of heart disease is increased after menopause than before. Women begin to outnumber men in terms of cardiovascular disease risk around ten years after menopause (Cooper *et al*. 1999).

2.1.6 Complications of IHD

IHD can lead to the following complications:

Pain in the chest (angina): During times of heavy demand, the heart may not receive enough blood if the coronary arteries narrow (especially during physical activity). As a result, angina (chest pain) or shortness of breath may ensue (Katz and Gavin 2019, Iaa *et al*. 2009).

A heart attack has occurred: If a blood clot forms as a result of a ruptured cholesterol plaque, the cardiac artery can become completely blocked, resulting in a heart attack.

Due to a shortage of blood supply, the heart muscle may be damaged (Katz and Gavin 2019, Iaa *et al*. 2009).

Heart failure: Because of restricted blood flow or damage to the heart caused by a heart attack, some areas of the heart are chronically oxygen and nutrients depleted. The heart's capacity to pump enough blood to meet the body's needs may diminish.

Irregular heartbeat (arrhythmia): It is a condition characterized by erratic heartbeat. Arrhythmia can be induced by a shortage of blood supply to the heart or by interfering with the electrical impulses of the heart (Katz and Gavin 2019, Iaa *et al*. 2009).

2.1.7 Geographical distribution of IHD

IHD is the world's most common cause of death. Myocardial infarction and ischemic cardiomyopathy are the clinical manifestations. Data is gathered from a variety of sources, including research studies, government statistics, hospital records, and other sources, in order to determine the regional distribution. And then these sources are analyzed. According to some research, the global infection rate between 1990–2017 was 126 million people, or 1655 people infected per 100,000, or 1.72% of the global population. And that this percentage will change in the coming years. Statistics have recorded that the incidence of infection in Eastern Europe is the highest (Khan *et al*. 2020).

Mortality and death rate for IHD is low in Western Europe, and it is also high and high in Northern Europe, Central Asia and the Middle East. In east Asia and Sub-Saharan Africa, it is relatively uncommon (Figure 2.4), (Moran *et al*. 2012). In addition, the family component plays a very important role in IHD (Nora *et al*. 1998).

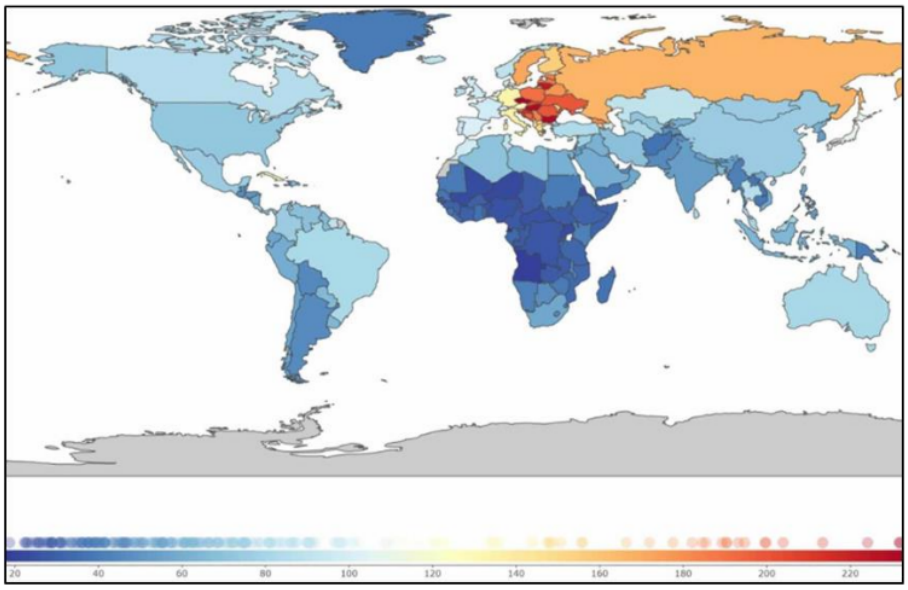

Figure 2.4 Geographical distribution for ischemic heart disease (Khan *et al*. 2020)

2.2 Relationship IHD with other Diseases

2.2.1 Diabetic and IHD

Diabetics with IHD are more likely to have poor cardiac outcomes. In diabetes and IHD patients, medication adherence is linked to better results. Quality improvement strategies to improve adherence to medication are required to make the most of cardiac preventive drugs (Ho *et al*. 2006).

Blood vessels can be damaged by having high blood sugar for a lengthy period of time, increasing the risk of heart disease and causing the body to not use sugar efficiently. Also, when the sugar level is high for a long period of time, blood vessels can become damaged, increasing the risk of heart disease and causing the body not to use sugar efficiently. High blood sugar levels are the beginning of the relationship between diabetes and heart disease. High levels of sugar damage the blood vessels over time, causing fatty substances to collect within them, causing hardening and hardening of the

arteries, impeding blood flow. A fatal heart attack or stroke occurs when blood reaches the heart and brain.

2.2.2 Hypertension and IHD

High blood pressure has been linked to strokes, ischemic heart disease, and renal failure. High blood pressure management and prevention are critical in the prevention of many disorders. According to recent evidence, hypertension is a significant factor in the pathophysiological condition or prodromal phases of many disorders (Kokubo and Iwashima 2015). Heart disease is caused by high blood pressure, and it is one of the leading causes of death connected with this illness. It can potentially cause serious health problems. IHD is caused by hypertension and keeping a healthy blood pressure is important for both primary and secondary prevention. In primary prevention, effective blood pressure (BP) control is suggested (Kharazmi-Khorassani *et al*. 2019).

2.2.3 Hypothyroidism and IHD

In the younger population, subclinical hypothyroidism (SCH) is only linked to an elevated risk of ischemic heart disease (both prevalence and incidence) and cardiovascular mortality. Furthermore, some evidence suggests that an increased vascular risk is only seen in persons with SCH who are younger (Razvi *et al*. 2008). According to several research, subclinical hypothyroidism is linked to ischemic heart disease and may affect men's all-cause mortality.

2.3 Signs and Symptoms

Ischemic heart disease lowers blood flow to the heart's coronary arteries, which transport oxygen. This decrease in blood flow can cause a variety of symptoms, which vary in intensity from person to person. Chest pain that happens on a daily or irregular basis is a symptom of IHD. Chest pain, pressure, or shortness of breath are some of the symptoms:

- Rest or medication can help you feel better.
- Chest pain can spread to the arms, back, and other regions of the body.
- You can get gassiness or indigestion (more common in women).
- It happens when the heart has to work harder, which commonly happens during physical activity.
- It usually only takes a few minutes (five minutes or less).
- In some cases, IHD can be life-threatening. The following are some of the signs and symptoms:
- Pain in the chest, on the left side of the body, in most cases (angina).
- Moistened skin
- Nausea, whether or not it is accompanied with vomiting.
- Neck and jaw pain.
- Shortness of breath or tachypnea (rapid breathing).
- Shoulder or arm pain is a common ailment (IHD 2020).

2.4 Diagnosis

2.4.1 Radiological examinations

- Electrocardiography (ECG)
- Treadmill Test-TMT: Patients with symptoms but normal ECG patterns may benefit from exercise testing (Treadmill Test-TMT).
- Echocardiogram
- Ventriculogram performed during a cardiac catheterization
- Gated SPECT: is a nuclear medicine imaging technique.
- Magnetic Resonance Imaging (MRI) of chest.

2.4.2 Laboratory examinations

Cardiac biomarkers:

- Creatine phosphokinase (CPK)
- Muscle–Brain creatine kinase (CK-MB)
- Troponin – T (TnT)
- Troponin – I (TnI)

Other cardiac biomarkers: Elevated in MI but non-specific:

- Aspartate aminotransferase (AST or GOT)
- lactate dehydrogenase (LDH)
- Myoglobin (Mb)
- N – terminal pro b – type Natriuretic Peptide (NT – proBNP or BNP)
- High sensitive C – reactive protein (hs-CRP)
- D-dimer
- Lipid profile test
- Total cholesterol
- Triglycerides Test
- High Density Lipoprotein (HDL)
- Low Density Lipoprotein (LDL)
- Very Low Density Lipoprotein (VLDL) (Bozbas *et al*. 2006, AlSaad *et al*. 2020, Aydin *et al*. 2019).

After angina pectoris develops into acute myocardial infarction, the levels of cardiac enzymes can be observed according to the time of injury, as shown in the following chart (Figure 2.5).

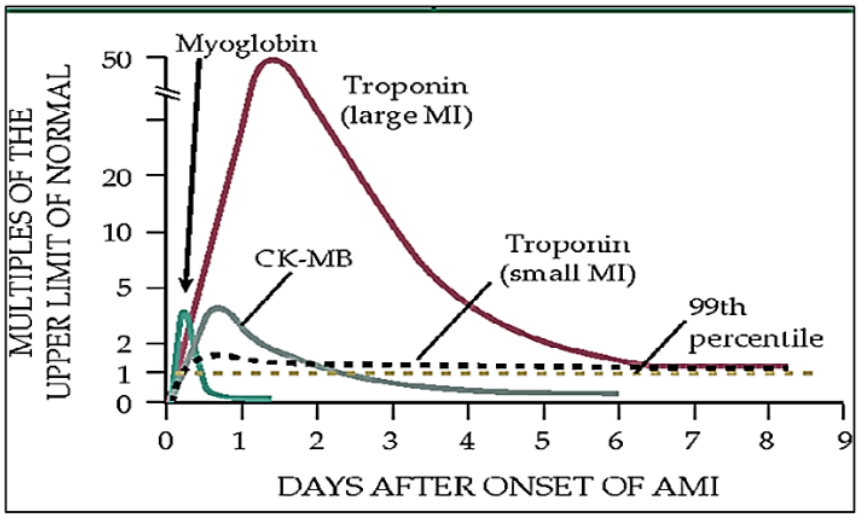

Figure 2.5 Time after heart infarction (Pandey *et al*. 2011)

2.5 Treatment

- Acetylsalicylic acid (Aspirin); Taking an aspirin or other blood thinner on a daily basis can help avoid blood clots, which can lead to blocked coronary arteries. In accordance with the doctor's recommendations.
- Nitrates; These drugs help to open up arteries and increase blood flow to and from the heart. helped in the improvement of cardiac blood flow.
- Beta blockers; These medications act by relaxing the heart muscle, decreasing the heartbeat, and lowering blood pressure, enabling more blood to circulate freely to the heart.
- Calcium-channel blocker; These drugs enlarge and relax blood arteries, allowing more blood to flow through the heart. Calcium channel blockers function by slowing down the heart's workload.
- Cholesterol-lowering medications; These drugs reduce the amount of primary material that builds up in the CAD.
- Angiotensin-converting enzyme (ACE) inhibitors; These drugs work by relaxing blood arteries and lowering blood pressure. The doctor may prescribe an ACE inhibitor if the patient has excessive blood pressure, diabetes, or myocardial

ischemia. As a result, ACE inhibitors may aid people with heart failure or a heart that does not pump blood properly.

- Anti-ischemic agents such as ranolazine (Ranexa); This drug relieves angina by relaxing the coronary arteries. Other angina drugs, such as calcium channel blockers, beta blockers, or nitrates, may be used in conjunction with ranolazine (McCormack *et al*. 1996).

2.6 Osteopontin (OPN)

2.6.1 Define of OPN

Osteopontin (OPN) is a protein present in the bones also referred to as bone sialoprotein I (BSP – 1 or BNSP). OPN is a 314-amino-acid glycoprotein that is extensively phosphorylated, has acidic characteristics, and contains a lot of aspartic acid. OPN is a multifunctional protein that plays a role in a variety of conditions, including cardiovascular disease, cancer, diabetes, kidney stone disease, inflammatory processes, biomineralization, cell viability, and wound healing. It regulates osteoclast function and affects CD44 receptors through IL–3, IL–10, IL–12, IFN, interleukin αvB3, nuclear factor – kappa B (NF-kB), macrophages, and T – cells, all of which are regulated by osteopontin (Icer and Gezmen-Karadag 2018).

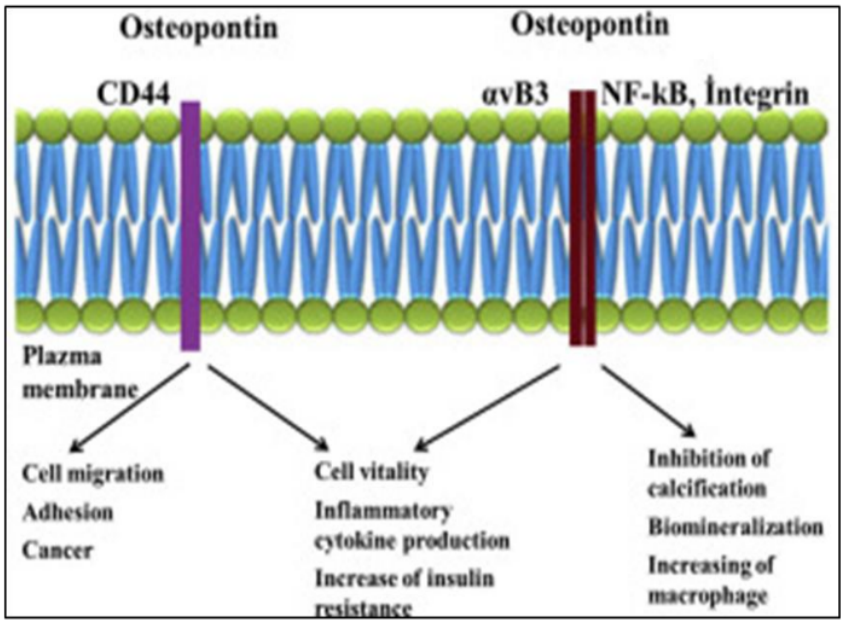

Figure 2.6 Osteopontin (OPN) (Icer and Gezmen-Karadag 2018)

2.6.2 Structure OPN

The structure of human osteopontin protein is displayed in Figure 2.7:

- "The amino acid sequence of the aspartate domain Hydroxyapatite is bound by Asp86-Asp89".
- "RGD is an amino acid sequence that starts with R and ends with αVβ3, αVβ1, αVβ5 and α5β1 integrins bind to Arg159-Asp159".
- "SVVYGLR sequence amino acid sequence Ser162-Arg168-binds α9β1 and α1β1 integrins".
- "Thrombin cleavage site-amino acid sequence Arg168-Ser169-displays RGD sequence".
- "Calcium binding domain-amino acid sequence Asp216-Ser228-calcium-binding".
- "Heparin-binding domain-amino acid sequence Asp290-Ile305 - mediates CD44v3 binding" (Mirzaei *et al*. 2018).

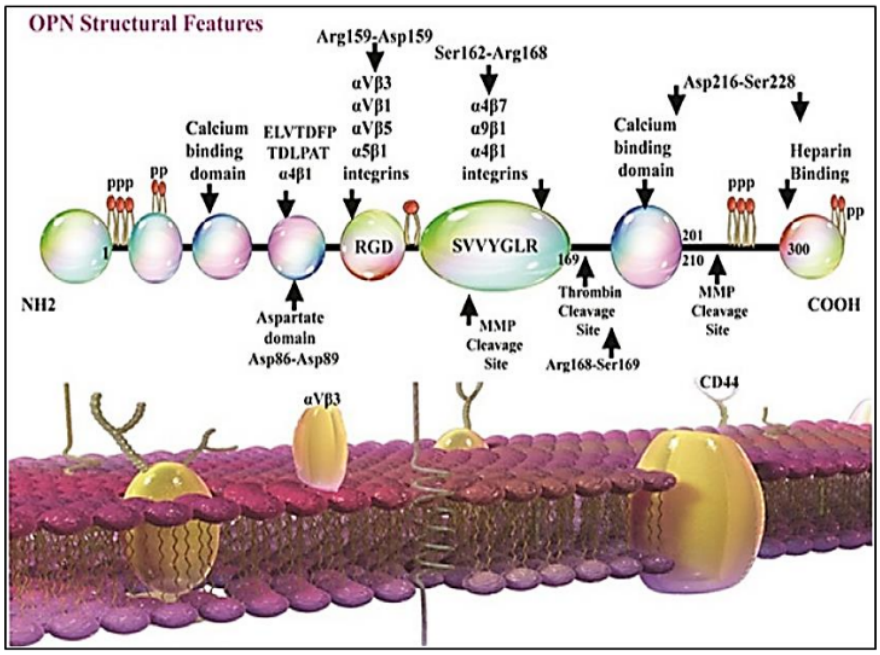

Figure 2.7 The human osteopontin protein's structure (Mirzaei *et al*. 2018)

2.6.3 Function of OPN

It is an osteogenic sialoprotein that connects osteoclasts to the mineralized bone matrix in addition to being an osteoclast matrix protein. OPN is a flexible protein that interacts with a number of cancer-related receptors and signaling pathways. It has a role in a number of biological processes that affect the immune and circulatory systems. Immunology, cell adhesion, migration, and tumor formation are all biological processes that it plays a role in. In a variety of cancer types, including brain, lung, kidney, liver, bladder, breast, esophagus, stomach, colon, pancreatic, prostate, and ovarian cancers, it also plays a critical in the growth of cancer cells, angiogenesis, and metastasis, as well as the chemical mechanisms that drive these actions. (Figure 2.8). It may have an impact on tumor formation, cancer treatment, and the discovery of new anticancer drugs (Zhao *et al*. 2018).

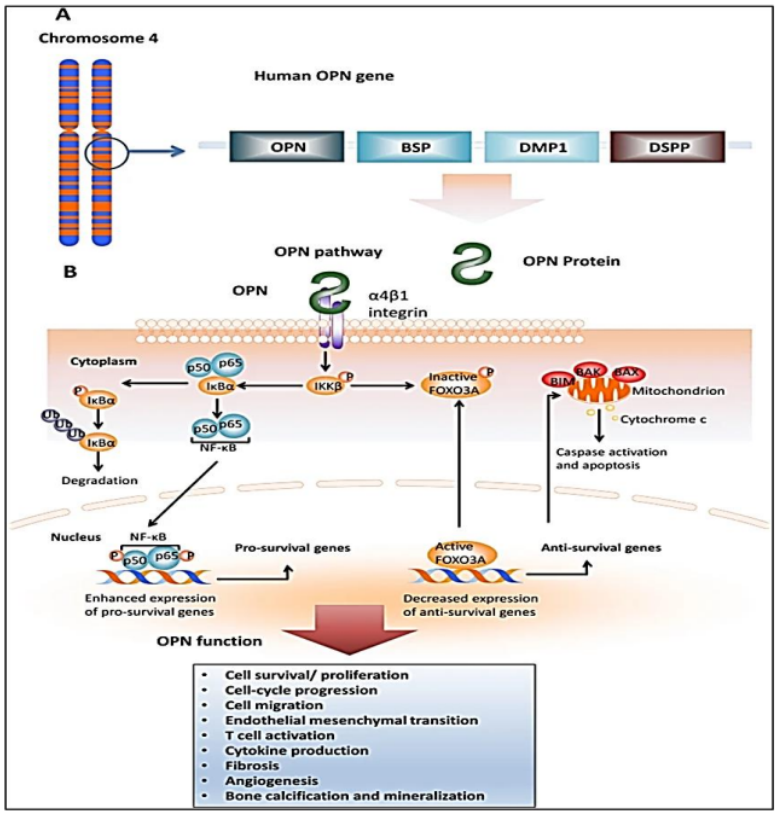

Figure 2.8 Function of osteopontin (Zhao *et al*. 2018)

2.6.4 Role of osteopontin in IHD

There are studies that show that the existence of ischemic heart disease affects the value of the OPN level, and gender plays a significant influence in this. Elevated OPN levels are a critical indicator of heart failure or one of the stratification criteria for hyposystole (Coculescu *et al*. 2019). There is also evidence for a role for OPN during ischemic limb revascularization in organisms (Duvall *et al*. 2008).

2.7 Blood Lipid Composition IHD

The truth about adipose tissue. Over 15 varieties of cholesterol – containing lipoproteins and four types of triglyceride – rich molecules exist, some of which are

exceedingly rigid, and all of which increase the risk of clinical cardiovascular disease. These many components must be present in the circulatory system in the proper proportions to lessen the chances of heart disease. Through the tests, it is possible to detect excess lipids in patients with fatty atherosclerosis, and thus reduce the risk of cardiovascular disease, using two simple methods to assess plasma lipid levels. After determining the ratio of total cholesterol to high – density lipoprotein cholesterol (HDL-C), plasma triglyceride concentrations should be measured. This will allow researchers to determine whether the primary cause of worry is low – density lipoproteins (LDL), high density lipoprotein (HDL – C), or triglyceride (TG) – rich molecules such beta-low-density lipoproteins (VLDL). Individuals with a higher lipid risk should be treated before, not after, coronary heart disease (CHD) is diagnosed (Castelli 1996).

There is also a study indicating the role of magnesium in ischemic heart disease, and through its deficiency leads to a change in the formation of fats in the blood, which increases atherosclerosis (Rasmussen *et al*. 1989).

Lipoprotein molecules carry fats like cholesterol and TG through the bloodstream. These include LDL, which carries the majority of blood cholesterol, VLDL, which carries the majority of triglycerides in the blood, and HDL, which carries the majority of triglycerides in the blood, and HDL, which carries the majority of TG in the blood. The most generally known risk factor for heart disease is high LDL – C. Excess triglycerides play a role in CAD as well also, low concentrations of HDL – C can also be a significant risk factor.

When HDL-C is insufficient, excess TG may be more essential. The relationship between total cholesterol and HDL is fascinating, and some individuals measure the ratio of total cholesterol to HDL, which in normal circumstances should be less than 5. It has long been known that blood cholesterol (and therefore LDL – C) is a significant risk factor for cardiovascular disease and other serious heart diseases. This link is thought to be a prognostic sign for IHD and other cardiac diseases, and it is thought to be causally connected.

According to several research, lowering the percentage of lipids in the bloodstream, such as cholesterol and LDL, using pharmacological or nutritional approaches, or both, reduces the risk of cardiovascular disease. As a result, an increase in blood cholesterol can be used as a predictor of future IHD and other heart illnesses (Law 1998).

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3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Laboratory equipments

Table 3.1 lists the instruments and equipment used.

Table 3.1 Instruments and apparatus have been employed

Instrument / Equipment	Company	Origin
BS-230 Clinical Chemistry Analyzer	Mindray	China
ELISA system reader	BioTek	USA
ELISA washer	BioTek	USA
Incubator	GEMINI	Germany
Printer	Epson	Japan
Autoclave	Hirayama	Japan
Centrifuge	Hettich	Germany
Micropipettes 5 – 50 μL	Cleaver	UK
Micropipettes 10 – 100 μL	Cleaver	UK
Micropipettes 100 – 1000 μL	Cleaver	UK
Refrigerator	Kelon	Korea
Water Distillater	GFL	Germany
EDTA	AFCOVAC	Jordan
Gel tube 6 mL	PUTH^{TM}	China
White tube	CITOTEST	China

3.1.2 Sample collection

The trial participants gave a total of 5 mL of venous blood. 2 mL were injected into anticoagulant tubes containing ethylene tetra-acetic acid EDTA to assess osteopontin and HbA1C. The remaining blood was collected in gel tubes and left for a short period in the laboratory until clotting, then placed in a device Centrifuge at 3000 revolutions per minute, and the serum was separated and placed in a white tube and frozen at a

temperature 20°C (until use). The second tube (gel tubes) used for lipid profile (TC, TG, HDL and LDL) and hs-CRP assays.

3.1.3 The study groups

Groups: The study is classified on the following groups:

- Group–A: Control group 60 people.
- Group–B: Involved 60 men with cardiac ischemia disease without diabetes mellitus. Age of patient: Their ages between 22- 70 year for analysis.

3.2 The Istatistic

The statistical analysis was carried out using SPSS 25 Version. Outliers were found in the data. All of the data were parametric, and the one-way ANOVA test was used to compare the groups. Comparison test to determine differences between specific groups. The standard deviation of the mean is used to represent the results (mean ± SD).

3.3 Methods

3.3.1 Human osteopontin examination by ELISA

Principle: This kit uses two types of high specific antibodies in a solid phase sandwich ELISA. As a coloring agent, Tetra Methyl Benzidine (TMB) is utilized (Chromogen). The amount of Human OPN present determines the intensity of the color.

Coating Antibody: Purify OPN Anti-Human (O – 17) IgG Affinity in Rabbits: The antibody binds to a part of the human OPN N–terminal region.

Labeled Antibody: Anti-Human OPN (10A16) Mouse IgG MoAb Fab'–HRP: The antibody binds to human OPN on the right side of the thrombin cleavage site.

Preparation:

- Preparation of wash buffer: A can of Wash buffer is mixed with 1950 mL distilled water to make a total volume of 2000 mL.
- Preparation of Labeled antibody: Mix labeled antibody concentration with solution for labeled antibody so that it is ready to add 100 µL per well.
- Standard preparation: Only 0.5 mL distilled water should be poured into the vial of Standard, and it should be mixed gently and completely. Human OPN is present in this solution at a concentration of 640 ng/mL (9.850 pmol/L).
- Dilution of the Standard: For the dilution of the Standard, prepare 8 tubes. Fill each tube with 230µL of EIA buffer. Enter the following concentrations for each tube into the tube.

- Tube-1: 320 ng/mL (4,920 pmol/L)
- Tube-2: 160 ng/mL (2,460 pmol/L)
- Tube-3: 80 ng/mL (1,230 pmol/L)
- Tube-4: 40 ng/mL (615 pmol/L)
- Tube-5: 20 ng/mL (308 pmol/L)
- Tube-6: 10 ng/mL (154 pmol/L)
- Tube-7: 5 ng/mL (76.9 pmol/L)
- Tube-8: 0 ng/mL (Test Sample Blank).

Fill tube–1 with 230 µL of Standard solution and gently mix it. Then, into the tube, pour 230 µL of tube–1 mixture. 2. Prepare 7 points of diluted standard between 320 ng/mL (4.920 pmol/L) and 5 ng/mL (76.9 pmol/L) by diluting two times standard solution in series. Tube–8 contains a 0 ng/mL test sample blank. As shown in Figure 3.1.

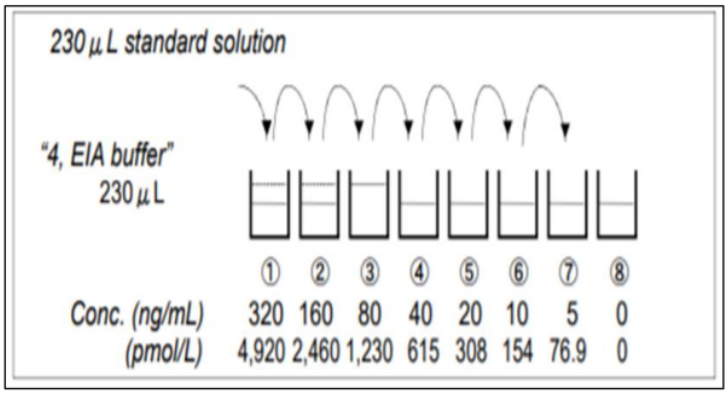

Figure 3.1 Perperation of standards

- Dilution of the test sample: If necessary, dilute the test sample with EIA buffer or PBS.

Procedure: Before using, bring all reagents to room temperature for around 30 minutes. Before using, stir gently and thoroughly. It must be assured that the reagents' quality does not deteriorate. The standard curve must be created at the same time as the test samples are being measured.

Calculate the result: Before planning, all data, including standards and unknown samples, are subtracted from the absorbance of a blank test sample. On logarithm graph paper, the subtractive absorbance of the standards versus the standard concentration is shown. The standard curve is then created by drawing the best smooth curve over these points. The concentrations of unknown samples are then calculated using the standard curve.

3.3.2 Examination of HbA1C

Principle: The concentration of hemoglobin is measured at a certain wavelength in the first reaction, while the reaction protease produces fructosyl dipeptides from the N-terminus amino groups of the beta-chain of HbA1c in the second. In the second step,

fructosyl peptide oxidase (FPOX) produces hydroperoxide from fructosyl dipeptides, allowing the sodium salt of 10-(carboxymethyl aminocarbonyl)-3,7-bis (dimethyl amino) phenothiazine to develop a color in the presence of peroxidase. HbA1C is calculated by measuring the change in absorbance. Using the combined assay findings for hemoglobin and HbA1C percent, the method calculates and expresses HbA1c percent.

Procedure: To perform the HbA1C test, the tube containing working samples is placed in the CS-230 device, which is a fully automated biochemistry device. After a quarter of an hour, the results are ready.

3.3.3 Examination of total cholesterol

Principle: The principle of examination of the cholesterol are illustrated in Equation (3.1), Equation (3.2), and Equation (3.3).

$$\text{Cholesterol ester} + H_2O \xleftrightarrow{\text{CHE}} \text{Cholesterol} + \text{Fatty acid} \quad (3.1)$$

$$\text{Cholesterol ester} + H2O \xleftrightarrow{\text{CHO}} \text{Cholestenone} + H_2O_2 \quad (3.2)$$

$$2H_2O_2 + 4\text{-Aminoantipyrine} + \text{Phenol} \xleftrightarrow{\text{POD}} \text{Quinonimine} + H_2O_2 \quad (3.3)$$

Cholesterol ester is catalyzed to yield H_2O_2 by the catalysis of CHE and CHO, which oxidizes 4-Amino antipyrine with phenol to form a colorful dye of quinonimine. The increase in absorbency is related to the level of cholesterol in the blood.

3.3.4 Examination of triglyceride

Principle: the principle of examination of triglyceride are illustrated in Equation (3.4), Equation (3.5), Equation (3.6), and Equation (3.7).

$$\text{Triglycerides} + 3H_2O \xrightarrow{\text{Lipase}} \text{Glycerol} + \text{fatty acid} \quad (3.4)$$

$$\text{Glycerol} + ATP \xrightarrow{GK} \text{Glycerol-3-phosphate} + ADP \quad (3.5)$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{GPO} \text{Dihydroxyacetone Phosphate} + H_2O_2 \quad (3.6)$$

$$H_2O_2 + \text{4-Aminoantipyrine} + \text{4-Chlorophenol} \xrightarrow{POD} \text{Quinoneimine} + HCI + 2H_2O \quad (3.7)$$

Triglycerides are formed through a series of enzymatic catalysis steps including lipase, GK, and GPD. Is catalyzed to produce H_2O_2, which oxidizes 4- Aminoantipyrinel to produce quinoneimine, a colorful dye. The increase in absorption is related to the triglyceride concentration.

3.3.5 Examination of HDL-cholesterol

Principle: The princible of examination of HDL-cholesterol are illustrated in Equation (3.8), Equation (3.9), Equation (3.10), and Equation (3.11).

$$\text{LDL, VLDL, Chylomicrons} \longrightarrow \text{Cholestenone} + H_2O_2 \quad (3.8)$$

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2 \quad (3.9)$$

$$\text{HDL} \xrightarrow{\text{CHE + CHO}} \text{Cholestenone} + H_2O_2 \quad (3.10)$$

$$H_2O_2 + \text{HDAOS} + \text{4-aminoantipyrin} \xrightarrow{POD} \text{Quinonimine} \quad (3.8) \quad (3.11)$$

3.3.6 Examination of VLDL

After conducting analyzes for cholesterol, triglyceride, HDL and LDL, the value of VLDL is calculated by applying the following equation: VLDL= Cholesterol – (LDL + HDL).

3.3.7 Examination of hs-CRP by ELISA

Principle: The human hsCRP ELISA is a sandwich-based, ready-to-use solid-phase enzyme-linked immunosorbent test that takes 1 hour and 10 minutes to complete. The assay can be run in any batch size thanks to the efficient format of a plate with disposable single breakable wells. Microtiter wells coated with antibodies that recognize human hsCRP are used to incubate samples and standards. The peroxidase-conjugated antibody will bind to the human hsCRP that has been captured. The substrate, tetramethylbenzidine (TMB), will react with the peroxidase-conjugated antibody. The addition of sulfuric acid stops the enzyme process. A spectrophotometer is used to measure the absorbance at 450 nm. Plotting the absorbance (linear) vs the equivalent concentrations of human hsCRP standards yields a standard curve (log). For samples run concurrently with standards, the standard curve can be used to determine the human hs - CRP levels.

4. RESULTS AND DISCUSSION

4.1 Cardiovascular Disease Analysis

Ischemia is a condition in which blood flow is restricted or reduced in a part of the human body as a result of this condition. In addition, cardiac ischemia refers to a decrease in the flow of blood and oxygen to the heart muscle in the human body. Our study included 120 adults aged 22-70 years for analysis. Control group - A 60 people, while group B included 60 men with ischemic heart disease without diabetes mellitus.

4.2 Age and RBS

The results revealed that the mean age of patients in groups A and B was (36 ± 4.55; 54 ± 6.63 mg/dL respectively). While the control group was (91.6 ± 16.8, 207.8 ± 43.6 year respectively). Shown the study significant statistically between patients' group and controls group in age as shown in Table (4.1) and Figure (4.1) While results (81 ± 13.2; 91 ± 11.6 mg/dL respectively) showed there were significant differences of RBS between A and B group, as shown in Table (4.2) and Table (4.3) and Figure (4.2). Also, the study showed (21.3 ± 0.9; 30.6 ± 4.18 kg/m^2 respectively) there were significant differences of BMI between group-A and B, as shown in Table (4.3), Table (4.4) and Figure (4.3). With increasing age, the adjusted relative risk of IHD for systolic blood pressure, diastolic blood pressure, and smoking decreased dramatically (P <0.001) (Tate *et al*. 1998). Diabetes mellitus is becoming one of the most common and expensive chronic diseases in the world.

Table 4.1 Age (year) in patients and HCs

Groups	No	mean ± SD	Minimum	Maximum	P value
Group-A	60	36 ± 4.55	23.0	70.0	0.027[a]
Group-B	60	54 ± 6.63	28.0	71.0	0.031[a]
Total	120	63 ± 7.86	37	70.5	0.031[a]
a = Mean significant difference between Groups-A with Group-B.					

Diabetes mellitus (DM) and cardiovascular disease (CVD), which is the leading cause of morbidity and mortality in diabetic individuals, have a tight relationship (Leon and Maddox 2015).

Table 4.2 RBS (mg/dL) in patients and HCs

Groups	No	mean ± SD	Minimum	Maximum	P value
Group-A	60	81 ± 13.2	70.0	112.0	0.131[b]
Group-B	60	91 ± 11.6	73.0	121.0	0.131[b]
Total	120	86 ± 12.4	71	116.5	0.131[b]
b = Mean no significant difference between Groups-A with Group-B.					

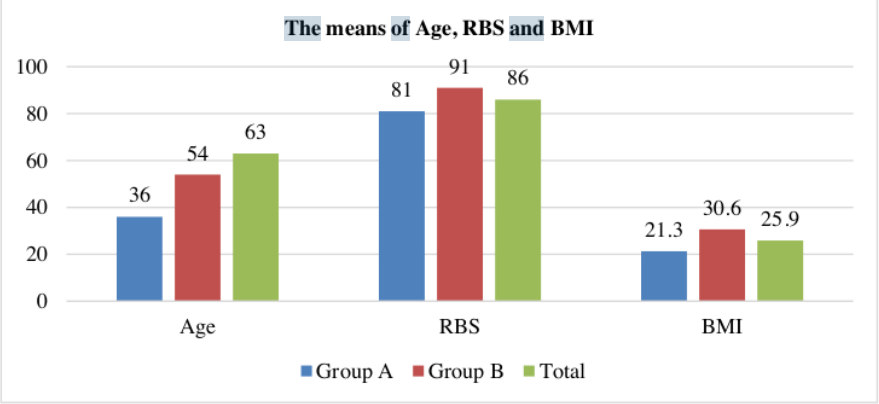

Figure 4.1 The means of age, RBS and BMI compared to controls group

4.3 Osteopontin (OPN)

In the same study showed (93.7 ± 14.6; 203.8 ± 33.8 respectively) there were significant differences of Osteopontin (OPN) between A and B group, as shown in Table (4.4), Table (4.5) and Figure (4.4). OPN is a secreted matricellular cytokine that communicates with integrin and CD44 receptors and has been linked to a variety of physiological and pathophysiologic events. OPN may fit into the Goldilocks paradigm for cardiovascular disease, in which acute elevations are favorable, minimize vascular calcification, and promote postischemic neovascularization, according to the data.

Chronic increases in OPN, on the other hand, have been associated in clinical studies to an increased risk of a serious adverse cardiovascular event, and OPN expression is a reliable predictor of cardiovascular illness (Lok and Lyle 2019).

Table 4.3 Osteopontin (OPN) in patient and HCs

Groups	No	mean ± SD	Minimum	Maximum	P value
Group-A	60	93.7 ± 14.6	93.0	123.0	0.034[a]
Group-B	60	203.8 ± 33.8	148.0	203.0	0.034[a]
Total	120	148.7 ± 24.2.2	74.0	163.0	0.034[a]
a = Mean significant difference between Group-A with Group-B.					

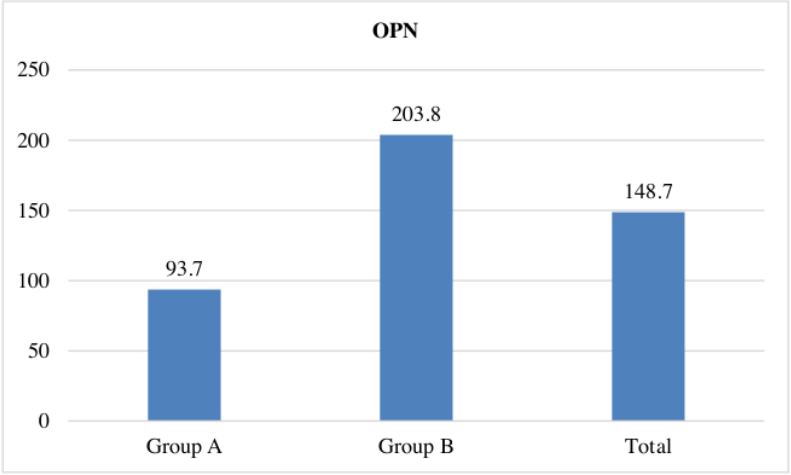

Figure 4.2 Shows the different between OPN mean group

4.4 HbA1c

In addition, Table (4.4) and Figure (4.3). The mean of HbA1c (%) has a significant difference between group-A (3.87 ± 0.75) as compared to the groups-B (5.91 ± 0.99) and the total was (4.89 ± 0.87) at P > 0.013HbA1c is linked to CVD such as carotid and CAD atherosclerosis, IHD, stroke, and hypertension. HbA1c causes dyslipidemia, hyperhomocysteinemia, and hypertension, as well as an increase in C- reactive protein, oxidative stress, and blood viscosity, all of which can lead to heart disease (Prasad 2018).

Table 4.4 HbA1C in patients and HCs

Groups	No	mean ± SD	Minimum	Maximum	P value
Group-A	50	3.87 ± 0.75	1.99	5.83	0.013[a]
Group-B	50	5.91 ± 0.99	4.65	8.09	0.013[a]
Total	150	4.89 ± 0.87	3.32	6.96	0.013[a]
a = Mean significant difference between Group-A with Group-B.					

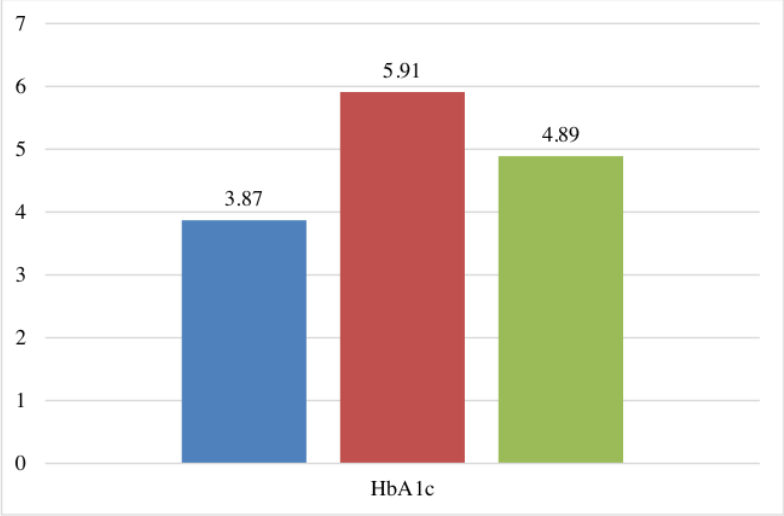

Figure 4.3 Shows the different between HbA1c mean groups

4.5 Total Cholesterol

Also, the results showed that non-significantly the mean total cholesterol of the group A was (171.0 ± 28.3 mg/dL) for control. While the patient's group was (273.0 ± 46.5 mg/dL). Shown the study significant statistically between patients' group and controls group in Tc as shown in Table (4.5) and Figure (4.4). There are now over 200 risk factors for CVD. Aberrant lipids, which comprise more than 15 different types of cholesterol – containing lipoproteins and four different types of triglyceride-rich particles, some of which are particularly atherogenic, high blood pressure, and cigarette smoking are the three most important (Castelli 1996). There was evidence of a statistically significant relationship between greater lipid levels and an increased future risk of CVD (Forbes *et al*. 2016).

Table 4.5 TC in patients and HCs

Groups	No	mean ± SD	Minimum	Maximum	P value
Group-A	60	171.0 ± 28.3	136	248.0	0.062[b]
Group-B	60	273.0 ± 46.5	154.0	310.0	0.062[b]
Total	120	222 ± 37.4	145	279.0	0.062[b]
b = Mean non-significant difference between Groups-A with Group-B.					

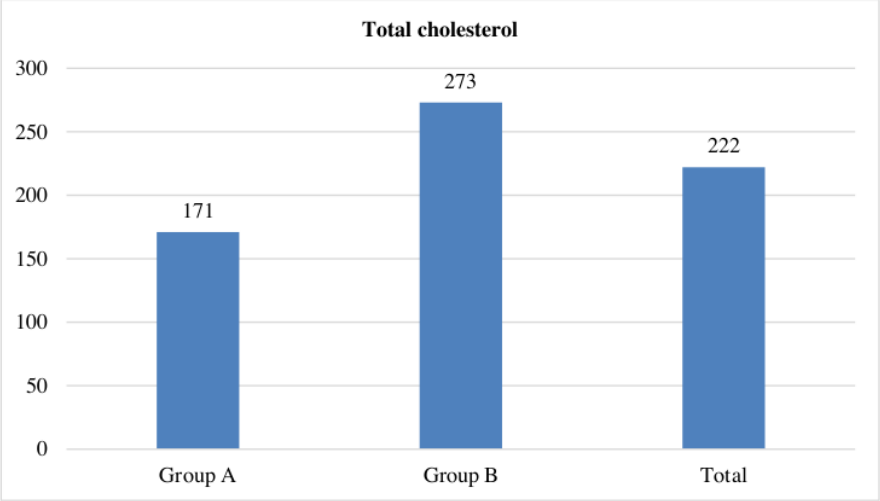

Figure 4.4 Shows the different between TC mean group

4.6 Triglyceride

As well as the mean of TG (mg/dL) in group A was (131.2 ± 26.3), which showed a significant difference with group B (201.7 ± 41.8) Table (4.6) and Figure (4.5) demonstrate this.

Table 4.6 TG in patients and HCs

Groups	No	mean ± SD	Minimum	Maximum	P value
Group-A	60	131.2 ± 26.3	73.0	181	0.041[a]
Group-B	60	272.3 ± 57.4	97.0	310	0.041[a]
Total	120	201.7 ± 41.8	85.0	245.5	0.041[a]
a = Mean significant difference between Groups-A with Group-B.					

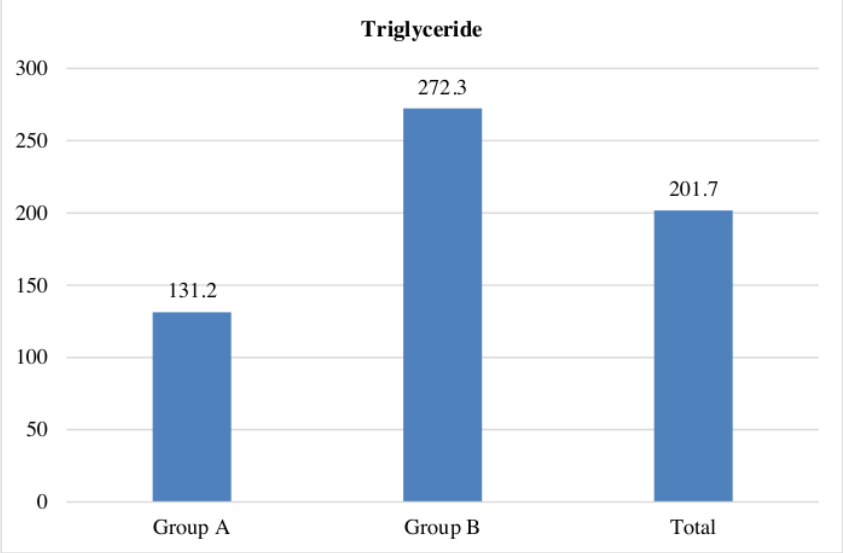

Figure 4.5 Shows the different between TG mean groups

4.7 HDL

Also, the mean of HDL (mg/dL) has a significant difference between group A (37.1 ± 8.03 control group) as compared to B group (68.0 ± 19.3), as seen in Table 4.7 and Figure 4.6.

Table 4.7 HDL in patients and HCs

Groups	No	mean ± SD	Minimum	Maximum	P value
Group-A	60	37.1 ± 8.03	34.0	64.0	0.020[a]
Group-B	60	68.0 ± 19.3	46.0	87.0	0.020[a]
Total	120	52.2 ± 21.1	14.0	94.0	0.020[a]
a = Mean significant difference between Groups-A with Group-B.					

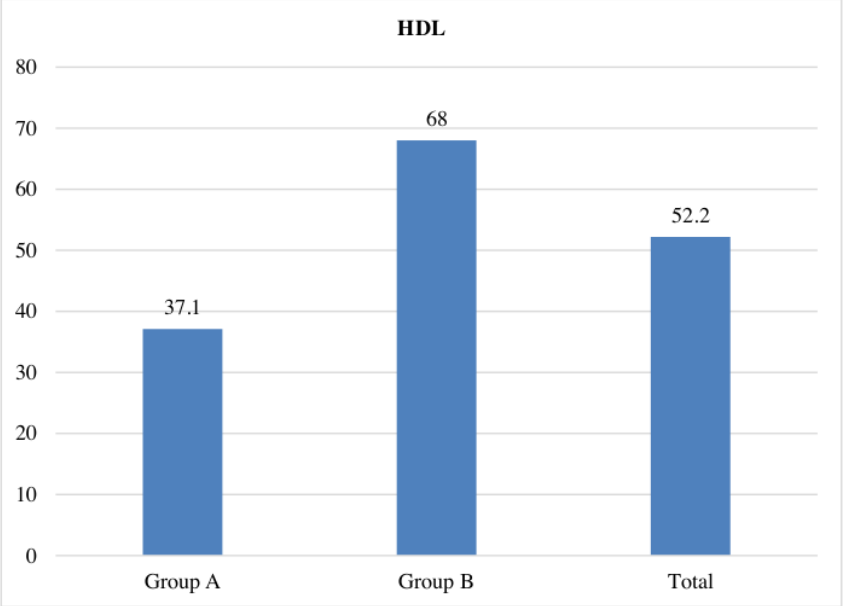

Figure 4.6 Shows the different between HDL mean groups

4.8 LDL

As well as the mean of LDL (mg/dL) in group A was (91.8 ± 26.1), which showed a significant difference with group B (171.9 ± 53.4) and significant difference with group B (Patients group) (169.3 ± 35.8) as shown in the Table4.8 and Figure 4.7. The total cholesterol content per LDL particle was negatively associated with triglycerides and positively associated with LDL-C. On follow-up, a CVD event. In multivariable models adjusting for nonlipid CVD risk factors, LDL-P was related more strongly to future CVD in both genders than LDL-C, this conclusion was in line with the findings of a prior report (Cromwell *et al*. 2007).

Table 4.8 LDL in patients and HCs

Groups	No	mean ± SD	Minimum	Maximum	P value
Group-A	50	91.8 ± 26.1	77.0	107.0	0.048[a]
Group-B	50	169.3 ± 35.8	92.0	194.0	0.048[a]
Total	150	130.5 ± 56.8	84.5	150.5.0	0.048[a]
a = Mean significant difference between Groups-A with Group-B,					

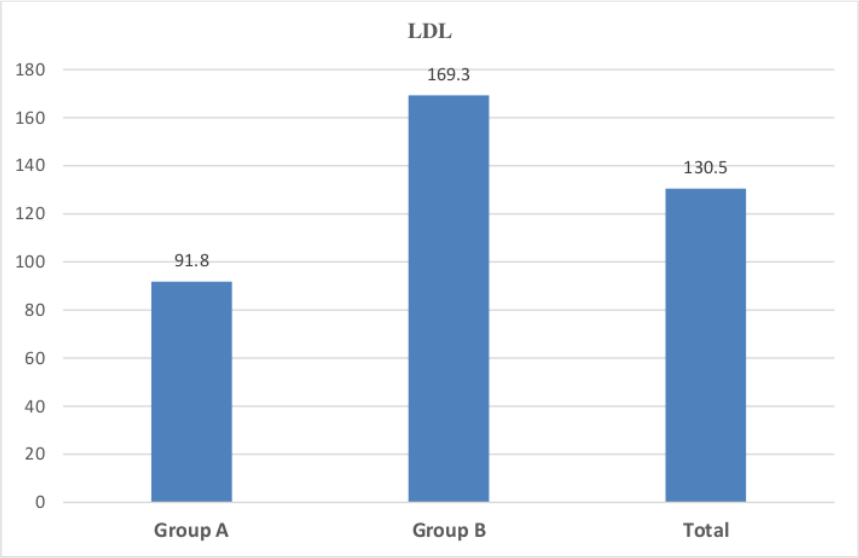

Figure 4.7 Shows the different between LDL mean groups

4.9 VLDL

The results are shown in Table (4.7) and Figure (4.9). The mean of VLDL in group-A was (26.2 ± 5.26), a significant difference between group-A with B groups (54.4 ± 11.4), Table (4.9) and Figure (4.9) demonstrate this.

Table 4.9 VLDL in patients and HCs

Groups	No	mean ± SD	Minimum	Maximum	P value
Group-A	50	26.2 ± 5.26	14.6	36.2	0.015[a]
Group-B	50	54.4 ± 11.4	19.4	62.0	0.015[a]
Total	150	40.2 ± 8.36	17.0	49.0	0.015[a]
a = Mean significant difference between Groups-A with Group-B.					

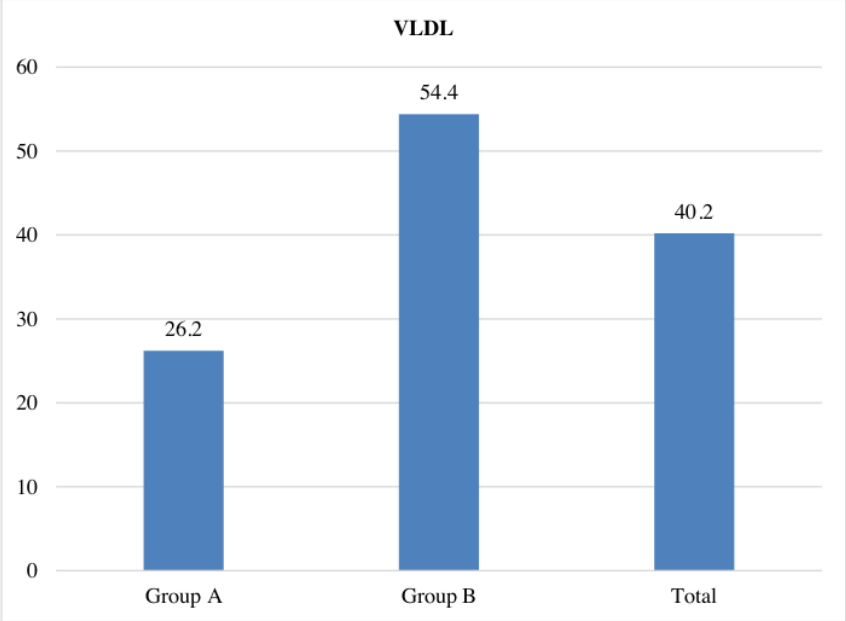

Figure 4.8 Shows the different between VLDL mean groups

4.10 hs CRP

Also, the mean of hs CRP (mg/dL) has a significant difference between group A (3.55 ± 2.39) as compared to B and C groups (6.68 ± 2.50) and (6.16 ± 2.00) respectively, Table (4.10) and Figure (4.9) illustrate this.

Table 4.10 hs-CRP in patients and HCs

Groups	No	mean ± SD	Minimum	Maximum	P value
Group-A	60	3.15 ± 2.48	1.19	8.93	0.036[a]
Group-B	60	5.62 ± 1.94	1.28	9.63	0.036[a]
Total	120	4.38 ± 2.21	1.23	9.28	0.036[a]
a = Mean significant difference between Group-A with Group-B.					

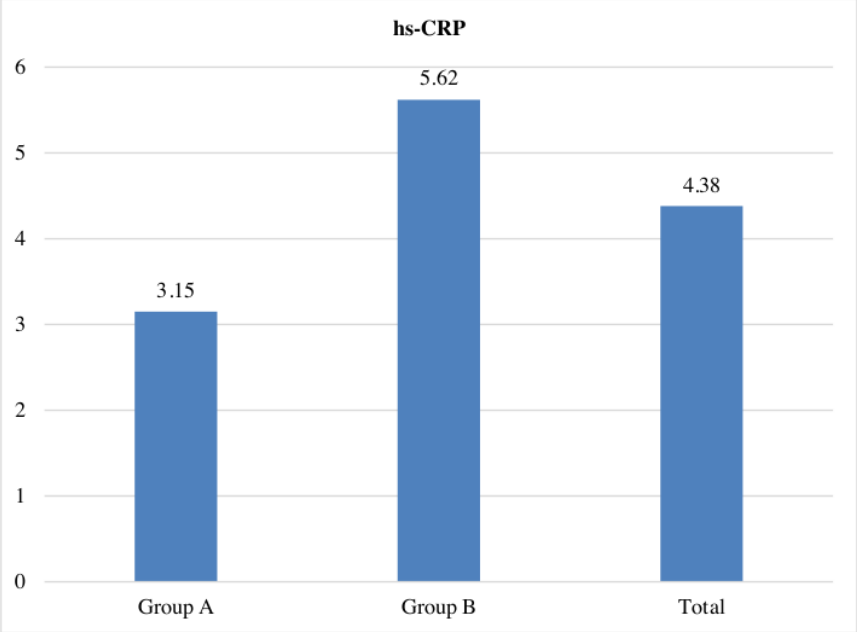

Figure 4.9 Shows the different between hs-CRP mean groups

4.11 Correlation between Osteopontin (OPN) with Studied Parameters

In this study, we find correlation between Osteopontin (OPN) with biochemical variables, as shown in above Tables. Results have shown, positively correlation between Osteopontin (OPN) with age, height, BMI, and FBS as shown in Table 4.12. However, this correlation was successful to reach a statistically significant value. Positively weakly correlation with weight at $P < 0.05$, this negatively trend was statistical significantly and cannot ignore ($r = 0.420$, $P < 0.05$), Table (4.11). As demonstrated in Table (4.11), there is a positive link between OPN, FBS, BMI, HbA1c, and Lipids at $P < 0.01$.

Table 4.11 Correlation between HOMA-IR and some variables in all case of T2DM

	Osteopontin (OPN)	
Variables	**r**	**P-Value**
Osteopontin (OPN) - Age (years)	0.154	N.S
Osteopontin (OPN) - Weight (kg)	0.361*	< 0.05
Osteopontin (OPN) – BMI (kg/M²)	0.313*	< 0.05
Osteopontin (OPN) - F.B.S (mg/dL)	0.138**	0.009
Osteopontin (OPN) - HbA1c (%)	0.079	0.281
Osteopontin (OPN) – TC (mg/dL)	0.254**	0.007
Osteopontin (OPN) - TG (mg/dL)	0.091	0.380
Osteopontin (OPN) – HDL.C (mg/dL)	- 0.326*	0.013
Osteopontin (OPN) – LDL. C (mg/dL)	0.294**	0.002
Osteopontin (OPN) – VLDL.C (mg/dL)	0.066	0.406
Osteopontin (OPN) – hs CRP (mg/dL)	0.041**	0.053

No asterisk; (P > 0.05);
**Highly significant at (P < 0.01);
*Statistically significant at (P < 0.05);
N.S.; non-significant

5. CONCLUSIONS AND RECOMMENDATION

5.1 Conclusions

Results have shown, positively correlation between Osteopontin (OPN) with age, height, BMI, and FBS as shown in Tables. Osteopontin (OPN) expression was increased in patients with Ischemia and CVD and correlated with disease activity. OPN may be a biomarker for the diagnosis of Ischemia and CVD. In patients with heart disease in Iraq, there was a direct positive association between OPN serum levels and hs-CRP when compared to a control group of the same ages, with r = 0.041** and P= 0.053; hence, these parameters may be employed as biomarkers for risk Ischemia and CVD patients. Osteopontin and hsC-RP play a role in Ischemia; therefore, these parameters may be used as biomarkers for risk Ischemia patients. Because of the high importance (correlation) shown by our study between the patient group (group-B) and control group (group-A), we can these variables be used to precise prediction of Ischemia patients. There are relationships of strength between troponin variables and some biochemical variables such as Age, hs-CRP, RBS and lipid profile. In the Ischemia group (group-B), the mean HbA1C, serum RBS and HDL, LDL, as well as the detection percentages of serum OPN and hs-CRP were considerably greater than in the control group (group-A). OPN, hs-CRP, HbA1C, RBS, and lipid profile levels in serum could provide further information on Ischemia patients' risk factors.

5.2 Recommendations

It is recommended for future researchers to conduct studies on the relationship of IHD with other diseases such as hyperthyroidism with its link to other cardiac enzymes, consisting of N–terminal pro b-type Natriuretic Peptide (NT–proBNP or BNP), Myoglobin (Mb), lactate dehydrogenase (LDH), Aspartate aminotransferase (AST or GOT), Muscle – Brain creatine kinase (CK-MB), Troponin–I and Troponin–T.

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