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EVALUATION OF APELIN AND SOME BIOCHEMICAL PARAMETERS IN CORONARY ARTERY PATIENTS IN MOSUL CITY

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EVALUATION OF APELIN AND SOME BIOCHEMICAL PARAMETERS IN CORONARY ARTERY PATIENTS IN MOSUL CITY

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June 2023

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ABSTRACT

EVALUATION OF APELIN AND SOME BIOCHEMICAL PARAMETERS IN CORONARY ARTERY PATIENTS IN MOSUL CITY

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Master of Science in Chemistry

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The present case control study was performed from September 2022until April 2023 at Çankırı Karatekin Üniversitesi/ Department of Biochemistry on 78 patients of different ages with heart disease (such as coronary artery disease also called coronary heart disease or heart disease or myocardial ischemia) who were diagnosed in Mosul hospitals and were compared with the control group, which included 67 samples of healthy people, when measuring the concentration of Apelin, it was found that there was a significant decrease in the concentration of Apelin in people suffering from heart disease compared with the control group in which the concentration of Apelin was within normal rates, as it acts as a protective system of the body. A group of biochemical variables were measured and the correlation relationship was statistically found between their concentrations and the concentration of Apelin and the results of the study were as follows A significant increase in the concentration of urea, creatinine and uric acid in the blood of people suffering from heart disease was found compared with the control group, which was within the normal concentrations of biochemical variables, where the correlation with the concentration of Apelin was inversely related, as the higher the concentration of these variables, in turn, the lower the concentration of Apelin and the greater its effect on heart disease, while the higher the concentration of Aeplin reduces the effects on the heart .It was also noted that there is a significant increase in both cholesterol and triglycerides compared to the concentration of Apelin in patients, and it was also found that there is a significant decrease in the concentration of high-density lipids, as well as there is a significant decrease in enzymatic antioxidants, such as catalase enzyme, compared with the concentration of Apelin in patients. While we found no significant differences in the concentration of albumin, the concentration of sodium and calcium salts and the enzyme ALT compared with the concentration of Apelin in the blood.

2023, 58 pages

Keywords: Coronary artery diseases, Apelin, Heart diseases, Antioxidants catalase

ÖZET

MUSUL ŞEHİRİNDEKİ KORONER ARTER HASTALARINDA APELIN VE BAZI BİYOKİMYASAL PARAMETRELERİN DEĞERLENDİRİLMESİ

Khubayb Ameer Hamdoon HAMDOON
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Haziran 2023

Bu vaka kontrol çalışması, Eylül 2022'den Nisan 2023'e kadar Çankırı Karatekin Üniversitesi/ Biyokimya Anabilim Dalı'nda kalp hastalığı (koroner kalp hastalığı olarak da adlandırılan koroner arter hastalığı veya kalp hastalığı veya miyokardiyal iskemi gibi) tanısı konmuş farklı yaşlarda 78 hasta üzerinde yapılmıştır. Musul hastanelerinde ve 67 sağlıklı insandan oluşan kontrol grubu ile karşılaştırıldığında, Apelin konsantrasyonu ölçüldüğünde, kontrol grubuna kıyasla kalp hastalığı olan kişilerde Apelin konsantrasyonunda önemli bir azalma olduğu bulundu. Apelin vücutta koruyucu bir sistem görevi gördüğü için konsantrasyonunun normal oranlarda olduğu gruptur. Bir grup biyokimyasal değişken ölçüldü ve konsantrasyonları ile Apelin konsantrasyonu arasında istatistiksel olarak korelasyon ilişkisi bulundu ve çalışmanın sonuçları şu şekildeydi: Acı çeken insanların kanındaki üre, kreatinin ve ürik asit konsantrasyonunda önemli bir artış Apelin konsantrasyonu ile korelasyonun ters orantılı olduğu biyokimyasal değişkenlerin normal konsantrasyonları içinde olan kontrol grubu ile karşılaştırıldığında kalp hastalığından kaynaklanan bir hastalık bulunmuştur, çünkü bu değişkenlerin konsantrasyonu ne kadar yüksekse, sırasıyla konsantrasyonu o kadar düşüktür Apelin ve kalp hastalığı üzerindeki etkisi ne kadar fazla ise, Aeplin konsantrasyonu ne kadar yüksek olursa kalp üzerindeki etkileri azaltır. Ayrıca hastalarda Apelin konsantrasyonuna kıyasla hem kolesterol hem de trigliseritlerde önemli bir artış olduğu ve hastalarda Apelin konsantrasyonuna göre yüksek yoğunluklu lipidlerin konsantrasyonunda önemli bir azalmanın yanı sıra katalaz enzimi gibi enzimatik antioksidanlarda da önemli bir azalma olduğu bulunmuştur. Kandaki Apelin konsantrasyonu ile karşılaştırıldığında albümin konsantrasyonunda, sodyum ve kalsiyum tuzlarının konsantrasyonunda ve ALT enziminde anlamlı bir fark bulmadık.

2023, 58 sayfa

Anahtar Kelimeler: Koroner arter hastalıkları, Apelin, Kalp hastalıkları, Antioksidanlar katalaz

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CONTENTS

LIST OF SYMBOLS

cm	Centimeter
dL	Desileiter
g	Gram
Kg	Kilogram
mg	Milligrams
mL	Milliliter
mmol	Millimall
nm	Nanometer
%	Percent

LIST OF ABBREVIATIONS

Alb	Albumin
ACE2	Angiotensin –converting 2
ApLN	Apelin
AF	Atrial fibrillation
Ca	Calcium
CVD	Cardiovascular disease
CAT	Catalase
Cho	Cholestrol
CAD	Coronary artery disease
CAMP	Cyclieadenosine monophosphate
ELISA	Enzyme-linked immunosorbent assay
EMT	Epithelial-mecsenchymal transition
GC	Gastric cancer
GSH	Glutathione
GPX	Glutathione peroxidase
HCC	Hepatocellular carcinoma
HDL-C	High density lipoprotein-cholestrol
GSSH	Human glutathione oxidized
LDH	Lactate dehydrogenase
LDL-C	Low density lipoprotein -cholertrol
Mg	Magnesium
MMPs	Matrix metalloproteinase
MVD	Microvessel density
PCI	Myocardial infarction
MI	Myocardial infraction
NHLBI	National heart, lung and blood institute
NADPH	Nicotinamide adenine dinucleotide phosphate
NO	Nitric acide
NSCLC	Non-small cell lung cancer
OS	Oxidative stress
ROS	Reactive oxygen species
Na	Sodium
SOD	Superoxide
TF	Tissue factor
TG	Triglyceride
U.A	Uric acid
VLDL	Very low density lipoprotein-cholestrol

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1. INTRODUCTION

The heart's pumping action moves blood from the veins to the arteries and then to the rest of the body (Eble *et al.* 2009). The heart is a 12 cm long, 9 cm wide, hollow, muscular organ in the shape of a pyramid that sits roughly in the center of the thorax. The liver's job is to transport substances including oxygen, hormones, regulating molecules, nutrients, and waste products from the body's cells to the bloodstream, and its weight may be anywhere from 230 to 280 grams in women and 280 to 340 grams in men. Over the course of a lifetime, the human body pumps around 300 million liters of blood (Norman and Litwack 1997). Five different kinds of blood vessels—arteries, arterioles, capillaries, venules, and veins—connect the heart to the rest of the body and provide blood to the organs and tissues that need it. These arteries are essential for controlling blood pressure and changing how much blood is delivered to the body's vital organs. Vasodilation is the process through which blood vessels expand, whereas vasoconstriction is the opposite process (Norman and Litwack 1997). Important roles in blood pressure control are played by peptides generated in the blood vessels that are either vasodilatory (such as salusins) or vasoconstrictive/vasodilatory (for example apelins (Suzuki *et al.* 2007, Chong *et al.* 2007, Watanabe *et al.* 2008). Resistance vessel dilation when.

Both reactions were predominantly mediated by nitric oxide (NO) after being infused into the human forearm (Japp *et al.* 2008). The vasodilation of the human splanchnic artery that is induced by apelin in vitro is dependent on the NO mechanism (Salcedo *et al.* 2007). When applied to human vascular smooth muscle in vitro, apelin causes a contraction (vasoconstriction, Katugampola *et al.* 2001, Maguire *et al.* 2009). In light of recent studies, this article will go through the past, present, and future uses of apelins, which are known to have vasoconstrictive effects on the cardiovascular system.

In 1998, Tatemoto and coworkers found the adipokinin apelin in stomach tissue (Tatemoto *et al.* 1998). Apolins are generated in many other organs and tissues beyond the stomach (Pitkin *et al.* 2010, Castan-Laurell *et al.* 2011). These include the heart, lungs, kidneys, liver, adipose tissue, GI tract, brain, adrenal glands, and endothelium.

Apolin is found everywhere across the body, which suggests it performs a broad variety of functions. Almost every organ also has apelin and angiotensin-like 1 receptor (APJ) mRNA (Than *et al.* 2012). Similar to the distribution of angiotensin II and type 1 receptors, apelin and its receptor, the APJ, are widely dispersed throughout the body. Furthermore, despite the structural similarities between these receptors, their binding receptors are distinct. An angiotensin 1 receptor-like receptor gene was discovered in 1992. This novel receptor was first dubbed the APJ receptor (angiotension-like 1 receptor) due to the lack of a suitable ligand. The G-coupled receptor gene for the APJ receptor, a protein of 377 amino acids and 7 transmembrane domains, is located on chromosome 11p. Apolin was first identified (by Than *et al.* 2012) after the APJ receptor was found, apelin exerts its juxtacrine, paracrine, autocrine, etc. physiological effects through this route (Castan-Laurell *et al.* 2011).

1.1 Aim of Study

The correlation between Apelin and heart disease. There is evidence that people with coronary artery disease have lower levels of Apelin and antioxidants, which may raise the risk of cardiovascular complications. The apelin system is a general physiological regulator, and it plays a key role in cardiovascular physiology. Apolin regulates vascular tone, blood pressure, inotropy, angiogenesis, and hemostasis in the cardiovascular system.

2. LITERATURE REVIEW

2.1 Cornoary Artery Disease

Over a century later, in 1772, Heberden provided a clinical description of angina (Heberden 1772) but it wasn't until nearly another century later that pathologists started to pay attention to the coronary arteries and define thrombotic occlusions in addition to "ossification." For decades thereafter, however, medical professionals missed the link between these discoveries and the signs of myocardial ischemia. Cardiovascular physiologists initially observed "quivering" of the ventricles and rapid mortality due to coronary artery occlusion in the dog around the end of the 19th century (McWilliam 1889, Porter 1893). Clinical medicine, pathology, and physiology are three of the greatest areas of medical knowledge, and they all developed independently yet simultaneously. Myocardial infarction, as determined by pathologist Ludvig Hektoen in 1879, is caused by coronary thrombosis "secondary to sclerotic changes in the coronaries." The (Hektoen 1892) Acute myocardial infarction was established at autopsy in five cases documented by two Russian doctors with pathology training in 1910.For example: In 1919, electrocardiography was being utilized for the diagnosis of this illness, which was first stressed as a therapy by James B. Herrick (Herrick 1912). (Idem 1919) Until the middle of the twentieth century, these methods were the gold standard for treating individuals who had suffered a myocardial infarction.

2.2 Coronary Risk Factors

Acute myocardial infarction, which often affected seemingly robust men in their forties and fifties at the height of their productive years, was revolutionized by two important advances in the 1960s. In 1948, with the help of experts in clinical cardiology, biostatistics, and epidemiology, the National Heart Institute (later renamed the National Heart, Lung, and Blood Institute, NHLBI) launched the Framingham Heart Study. The researchers set out to learn the causes of cardiovascular illness by analyzing the habits of Framingham, Massachusetts locals.

Their original publication, "Factors of Risk in the Development of Coronary Heart Disease," (Kannel *et al.* 1961) indicated that hypertension and high cholesterol were associated with an increased risk of ischemic heart disease and acute myocardial infarction. Women, in contrast to men, were more likely to get a myocardial infarction later in life. Since the National Heart, Lung, and Blood Institute (NHLBI) implemented nationwide initiatives to educate doctors and the public on the significance of treating risk factors including high blood pressure and cholesterol, the age-adjusted mortality rate from cardiovascular disease has declined substantially (NHLBI 2010).

2.3 Coronary Care Units

If a person with acute myocardial infarction was lucky enough to make it to a hospital before dying, they would have been put in a bed distant from the nurses' stations before 1961. A deadly tachyarrhythmia was likely the cause of death, since patients were often discovered in their beds. Death really occurred in the hospital around 30% of the time. Patients hospitalized with acute myocardial infarction now have a 50 percent lower risk of dying while in the hospital because to the establishment of the coronary care unit (Julian 1961), which provides continuous monitoring of the ECG, closed-chest cardiac resuscitation, and external defibrillation.

2.4 Cardiac Catheterization

Coronary arteriography was first performed in 1958, made possible by cardiac catheterization. Reference: (Sones and Shirey, 1962) Clinicians were able to learn more about the natural history of coronary artery disease by using this imaging approach in conjunction with left ventriculography. Diagnostic procedures like coronary angiograms and left ventriculograms.

Instrument that established the baseline for coronary revascularization surgery by determining pump function and artery structure. Surgeons, engineers, cardiologists, anesthesiologists, and hematologists all worked together to perfect the art of open-heart surgery. As reported by Gibbon (1954) Although Andreas Grüntzig is often regarded as the forefather of percutaneous interventional cardiology (Grüntzig *et al.* 1979), the area of invasive cardiology did not take long to develop, building on the groundwork laid by Dotter and Judkins. Balloon angioplasty was the first method employed, then bare metal stents were implanted, and now drug-eluting stents are used to avoid coronary restenosis (Serruys 2002).

2.5 Natural History and Prognosis

High blood pressure, high cholesterol, diabetes, inactivity, obesity, smoking, and a family history of coronary artery disease (CAD) all decrease a patient's prognosis. Patients with SCAD should have a prognostic assessment as part of their management, as suggested by a recent research (Montalescot *et al.* 2013). A better prognosis may be achieved, on the one hand, by precisely identifying persons with more severe forms of disease and providing them with more intensive examination and, perhaps, intervention (such as revascularization). On the other side, identifying patients who have a milder type of illness and a better prognosis may help save them from invasive and non-invasive diagnostics and revascularization operations they don't need. Patients with SCAD have a favorable prognosis, with yearly mortality rates estimated between 1.2% and 2.4% in a mixed population and an annual incidence of cardiac death between 0.6 and 1.4% (Boden *et al.*. 2007, Chung *et al.*. 2011 , Frye *et al.*. 2009 , Henderson *et al.*. 2003 , Poole-Wilson *et al.*. 2004 , Steg *et al.*. 2012).

2.6 Lifestyle and Pharmacological Management

The treatment of CAD focuses on alleviating symptoms and improving prognosis. Patients with coronary artery disease (CAD) may benefit from lifestyle modifications, control of CAD risk factors, pharmacological therapy supported by evidence, and patient education (Perk *et al.* 2012, Reiner *et al.* 2011) are only two of several research that have shown that It is recommended that people stop smoking, eat healthily, get regular exercise, and keep their weight, blood lipids, blood pressure, and blood glucose levels in check. Smoking is associated with a 36% reduction in mortality after a myocardial infarction, according to a recent research (Montalescot *et al.* 2013), and should be avoided at all costs for the prevention of cardiovascular disease. Piepoli *et al.* (2010) is the cited source. The risk of cardiovascular disease may be reduced by Ideal body mass index is less than 25 kg/m2. Patients who have been diagnosed with CAD and are physically active on a regular basis have a reduced risk of cardiovascular disease-related morbidity and mortality. Aerobic exercise training at a moderate to vigorous level, at least three times per week for at least thirty minutes per session, is recommended for patients with a history of acute myocardial infarction (MI), coronary artery bypass grafting (CABG), percutaneous coronary intervention (PCI), stable angina pectoris, or stable chronic heart failure (Montalescot *et al.*. 2013).

Lipid guidelines suggest treating dyslipidemia with a combination of medication and lifestyle changes. The goal of treatment is to reduce LDL-C to less than 1.8 mmol/L (70 mg/dL) or, if that cannot be achieved, to reduce LDL-C by at least 50%. Glycated hemoglobin (HbA1c) levels should be kept between 6.5% to 6.9% (48 to 52 mmol/mol) on average and below 7.0% (53 mmol/mol) for each patient to limit the risk of CV issues and the aggravation of cardiovascular disease (Montalescot *et al.*. 2013).

2.7 The Pathophysiology of Chronic CA

Atherogenesis was formerly thought to be a cholesterol storage illness, but we now know that it involves a number of risk factors, such as blood and chemical communications sent between cells of the arterial wall. Inflammation's central participation in all phases of atherogenesis is a valuable organizing motif that arose initially from laboratory investigations and has since acquired credibility in the clinic (Libby 2002). Endothelial cells and monocytes release matrix metalloproteinases (MMPs) in response to oxidative, hemodynamic, inflammatory, and autoimmune signals. MMPs and their natural tissue inhibitors regulate vascular cell activation, proliferation, migration, and death as well as new artery development, geometric remodeling, healing, or destruction of extracellular matrix of arteries and the heart.(Libby 2000) Lipoproteins are bound to extracellular matrix components (especially proteoglycans), which increases their time spent in the intima and hence their susceptibility to oxidative modification and glycation (nonenzymaticc conjugation with sugars).To wit: (Williams and Tabas, 1998)By-products of lipoprotein oxidation and glycation, respectively, maintain a high level of inflammation.Tabas (1999), Berliner *et al.* (2001). In time, the lesion might calcify via mechanisms similar to bone formation.(Demer 2002) Besides growth, atherosclerotic lesions often undergo cell death (including apoptosis) after they have been established (Geng and Libby, 2002) When lipid-laden macrophages die, some of their tissue factor (TF) is shed into the surrounding tissue as a particulate substance.As reported by Bogdanov *et al.* (2003) Atherosclerotic plaques often have a lipid-rich "necrotic" core that forms when extracellular lipid accumulates in the intima and forms a solid mass.

2.8 The Therapy of Chronic CAD

Myocardial ischemia caused by flow-limiting stenoses has been the determining factor in CAD treatment until recently. Nitroglycerin, nitrates, -blocking agents, and calcium channel blockers are all examples of therapies that aim to lower the oxygen demands of the heart by modifying its rate of contraction, inotropic state, and loading conditions.Drugs that improve glucose utilization and reduce the wasteful oxidation of free fatty acids to produce energy are on the horizon. Revascularization procedures are able to effectively restore forward coronary artery blood flow in the vast majority of patients. Throughout the last century, advances in surgical and percutaneous revascularization have been among the most groundbreaking therapeutic innovations. Innovative methods of revascularization include the use of gene, protein, or cell therapy to stimulate arteriogenesis. In addition to addressing flow-limiting lesions, we must also focus on nonobstructive plaques.Traditional angiography is unable to distinguish between ischemia caused by permanent stenoses and that caused by dynamic blockage, and whether or not the rapid spread of lesions indicates a poor prognosis (Moise *et al.* 1984). Indeed, permanent stenoses don't appear to evolve gradually but rather in rapid bursts (Bruschke *et al.* 1989, Yokoya *et al.* 1999).

For both primary and secondary prevention, aggressive treatment of modifiable risk factors has been shown in a number of randomized controlled trials to reduce mortality, myocardial infarction (MI), stroke, and other cardiovascular events, as well as the need for revascularization. For every 1 mm Hg decrease in blood pressure, 2-3% of long-term risk of MI is reduced, for every 10% drop in LDL cholesterol, 25% of long-term risk of MI is reduced, and for every % decline in cardiovascular deaths and events, 25% of long-term risk of MI is reduced. Just as quitting smoking immediately lowers the risk of heart disease. Both metabolic syndrome and type 2 diabetes are linked to a 5- to 10-year decline in life expectancy and a 2- to 4-fold increase in the risk of cardiovascular death. The National Cholesterol Education Program Adult Treatment Panel III report has defined and recently refined guidelines for the primary and secondary prevention of atherosclerosis using risk scales that take into account blood lipids, modifiable and nonmodifiable nonlipid risk factors, and other emerging risk factors (Grundy *et al.* 2004).

2.9 Oxidative Stress

When in vivo oxidation exceeds antioxidant activity, a condition known as oxidative stress (OS) occurs, causing an inflammatory response characterized by neutrophil infiltration and elevated protease release. Overproduction of free radicals (OS) is a key factor in aging and illness (Elhady 2019). OS arises when free radical production exceeds the antioxidant protection system's capacity. A small amount of reactive oxygen species (ROS) plays an important role in maintaining vascular tension and participating in membrane receptor-mediated signal transduction (Masoumi *et al.* 2018), despite the fact that antioxidants like superoxide dismutase (SOD), glutathione reductase (GR), and vitamin E in vivo clear the body of ROS produced by prooxidants during normal metabolism. Elevated levels of reactive oxygen species (ROS) have been linked to a variety of adverse health effects, including DNA damage and cardiomyocyte mortality (el-Awady *et al.* 2011, Ferroni *et al.* 2011). Therefore, ROS buildup and oxidative damage may be slowed with the use of powerful antioxidants. Antioxidant enzymes prevent ROS from being produced uncontrollably by neutralizing highly reactive free radicals (Matés *et al.* 2000). Recent research has shown that APLN inhibits ROS in adipocytes by binding to and blocking the activity of APJ receptors.This shows that APLN has a significant role in mitigating ROS-induced oxidative stress in adipocytes and other tissues (Than *et al.* 2014). APLN has been proven to have anti-inflammatory and antioxidant properties in a number of investigations (Than *et al.* 2014, Zhou *et al.* 2016, Bircan *et al.* 2016) Gunes *et al.* 2018).

2.10 Oxidative Damage to Nuclic Acide

Oxidative damage to DNA can be initiated by ionizing radiation or photooxidation (UV light, visible light in the presence of photosensitizers), hydroperoxides, oxygen radicals or various other oxidizing agents (von Sonntag *et al.* 1981). The nature of the damage is often complex. For example, a variety of ra- dicals can be formed upon y-irradiation of DNA, but only the radical 1 with a free electron on C-4 leads to strand breaks under anoxic conditions. In the presence of oxygen, the radical-mediated strand scission is more complex and proceeds via peroxy radicals of the bases and sugars. Base alteration has been studied most intensively with thymine and guanine. Thymine glycol 2 or 5-(hydroxymethy1)uracil are formed from thymine (cf. (Decarroz *et al.* 1986). The base most readily lost upon y-irradiation or pho- tooxidation is guanine. Adenine and cytosine, on the other hand, appear to be more stable towards oxidation. Thy- mine dimers are also formed, and the chemical ad- ducts upon reaction with epoxides.

2.11 Oxidative Damage to Amino Acids and Proteins

The oxidation of amino acid side-chains in proteins has recently been recognized as being a potentially important signal in biological systems. Redox reactions involving thiol groups (oxidation and reduction) have several connections to oxidative stress. The oxidation of methionine to methionine sulfoxide and its subsequent enzymatic reduction back to methinine are both examples of reversible oxidation of reactive groups.However, amino acids may suffer permanent oxidative damage, such as the ring breakage seen in histidine and tryptophan (Abrams *et al.* 1986).

2.12 Oxidative Damage to Carbohydrates

As mentioned in Section 2, deoxyribose is prone to oxi dative degradation. This property has even been exploited for the development of an assay of the formation of free- radicals. In the thiobarbiturate assay, deoxyribose yields a degradation product that is almost identical to the product obtained with malndialdehyde. (Halliwell Gutteridge 1981) Polysaccharides such as hyaluronic acid can be de- graded by oxidative attack, superoxide dismutase was found to be capable of protecting hyaluronic acid against depolymerization in synovial flid (McCord 1974, GreenWald 1981) Proteoglycans may be subject to oxidative breakdown in a similar manne (GreenWald 1981).

2.13 Antioxidants

Antioxidants are substances that assist mitigate the negative effects of oxidative stress by neutralizing free radicals (FRs) or preventing further damage to substrates such proteins, lipids, carbohydrates, and DNA.The (Dekkers 1996) Both enzymatic (endogenous) and non-enzymatic (mostly dietary) antioxidants are at work in the body.(Powers, Lennon, &amp, 2000) All of these antioxidants may function either within or outside of cells. These enzymes include glutathione peroxidase (GPX), glutathione superoxide dismutase (SOD), and catalase (CAT). Non-enzymatic antioxidants include vitamins A (retinol), C (ascorbic acid), and E (tocopherol), flavonoids, thiols (including glutathione [GSH], ubidecarenone (ubiquinone Q10), uric acid, bilirubin, and ferritin), and micronutrients (iron, copper, zinc, selenium, and manganese). Exercise, training, nutrition, and aging all have an effect on endogenous antioxidant enzyme synthesis, which in turn is dependent on dietary intakes (vitamins and micronutrients).The (Dekkers 1996) Furthermore, exercise boosts FR synthesis, making the effectiveness of the antioxidant system crucial in sport physiology.

2.14 Biochemical Antioxidants

One of the requirements for aerobic life is the detoxication of reactive oxygen species, and the numerous lines of defense that have developed to do this constitute a genuine antioxidant defense system. All three layers of defense—prevention, interception, and repair—are in play to counterbalance the potentially dangerous responses triggered by oxygen metabolites. It consists of both the enzymatic system and the nonenzymatic scavengers and quenchers often referred to as antioxidants.

2.15 Nonenzymatic Antioxidants

Aα-Tocopherol (vitamin E,) is the most important lipid –soluble antioxidant . (Burton *et al.* 1983) its unque function in the membrane may be aided by the specific physicochemi- cal interaction between the phytyl residue and the fatty acid residues of the polyunsaturated phospholipids in the membrane.'"(Diplock and Lucy 1973) The proper insertion of the vitamin into the membrane increases its effectiveness by about 50-f0ld.(Cadenas *et al.* 1984) Ascorbic acid (vitamin C), together with glutathione, is the important antioxidant in the aqueous phase. It can react with the vitamin E radical (chromanoxyl) and thus can regenerate tocopherol in the membrane. Further as- pects of interest, e.g. in nutrition(chow and clin 1979)1 (normal requirement: 12 mg of vitamin E and 75 mg of vitamin C per day (wirth 1984)

2.16 Enzymatic Antioxidants

Superoxide dismutases and other hydroperoxidases including glutathione peroxidase, catalase, and other hemoprotein peroxidases have been the subject of much research and are among the most important enzymes. In general, they are distinguished by a high specific cellular content, organ and subcellular localizations that frequently overlap and complement one another, and a distinct metal participation, particularly copper, manganese, iron (heme), and selenium, in the catal- ysis. The prevalence of these antioxidant systems in nature demonstrates their critical role in protecting biological systems from the debilitating effects of reactive oxygen metabolites. Their distribution matters much for toxicity to certain organs.In a study, (Ishikawa *et al.* Some recent advances in our understanding of responses that play a supporting role in the body's anti-oxidant defenses will be discussed below. A new protein has been found and described as a selenoenzyme that shields membranes from oxidation and has GSH peroxidase activity toward phosphatidylcholine hydroperoxides.It has been shown (Ursini *et al.* 1985) Another novel lipid peroxidation inhibitor found in biological membranes is a GSH-dependent heat-labile component.As reported by McCay *et al.* (1976). Recent evidence suggests that this cytosolic protein is not a member of the family of GSH-dependent proteins (Gibson *et al.* 1985). Some specific cell types may use extracellular GSH. The basolateral membrane of intestinal epithelial cells contains an Na@-dependent GSH uptake system, so that exogenous GSH protects these cells from oxidative injury. (McCay *et al.* 1976) " other cell types cannot utilize GSH as such, but have to synthesize their GSH intracellularly from the constituent amino acids. It's important to recognize the significance of certain supplementary or supporting structures. Glutathione (GSH) may be oxidized to GSSG, a reactive sulfhydryl group, by a variety of radical and nonradical processes in cells. Thus, GSSG reduc- tase's catalyzed regeneration process of reduction to GSH may play a crucial role in antioxidant defense. Providing reducing equivalents for this enzyme is obviously crucial as well. Systems capable of producing NADPH are therefore also of interest.

2.17 Apelin

The apelin receptor (APJ) is a G protein-coupled receptor that was discovered to be activated by the endogenous peptide apelin (Tatemoto *et al.* 1998). The digestive tract of a cow was the first place where an apelin was found. Similar in structure to angiotensin receptors is the apelin receptor (APJ), which is encoded by the APJ human gene (APLNR). In contrast to the angiotensin receptors, which are activated by angiotensin peptides, the apelin receptor peptidase J (APJ) interacts exclusively with apelin (O'Dowd *et al.* 1993). Both proteins have a high degree of homology in their transmembrane domains (54% sequence identical). However, APJ has no effect on angiotensin II (Lee *et al.* 2000). Similar to angiotensin II, apelin has been demonstrated to serve as a substrate for the catalytic activity of ACE2 in in vitro investigations (O'Dowd *et al.* 1993) consensus sequences for palmitoylation, glycosylation, and cyclic AMP-dependent protein kinase phosphorylation have been reported in this apelin receptor. On chromosome X, specifically at position Xq25-26.1, was found to be the apelin gene (APLN) (Lee *et al.* 2000). A prepropeptide of 77 amino acids is encoded by this gene. Shorter peptides (apelin-17, -12, and -13) and the full-length apelin peptide (apelin-36) are produced by C-terminal cleavage of preproapelin by apelinase. The enzyme apelinase cleaves preproapelin at its N-terminus to generate the full-length apelin peptide apelin-36 (Habata *et al.* 1999). High quantities of apelin peptides were found in cow milk and colostrum. Evidence from an extensive expression analysis suggests that preproapelin mRNA and APJ mRNA are expressed in very similar patterns throughout the same tissues. The similarity between the two mRNAs suggests a possible functional relationship between preproapelin and APJ in a variety of organs, where both are expressed and regulated (Lee *et al.* 2000). Expression of rat preproapelin and APJ mRNA was found in the central nervous system, reproductive organs, gastrointestinal tract, kidneys, and fetuses (O'Carroll *et al.* 2000). The spleen and liver of rats had the highest amounts of apelin mRNA, while the heart, lungs, and breasts all contained significant amounts (Kawamata *et al.* 2001). An immunohistochemistry analysis revealed abundant quantities of apelin-17 in chondrocytes, endothelial cells, the heart, the skin, the brain, the spleen, the thymus, and the lungs. Skeletal muscle was shown to have much greater apolin expression than any other tissue tested. Apolin-17 has been detected in low concentrations in liver, pancreas, and kidney (De Falco *et al.* 2002). The adipocytes also showed increased apelin expression. Adipokines, such as apol, are hormone-like substances released by fat cells. Tumor necrosis factor-alpha and interleukin-6 are examples of pro-inflammatory classical cytokines, monocyte chemoattractant protein-1 is an example of a chemokine, and plasminogen activator inhibitor and angiotensinogen are examples of adipokines involved in vascular homeostasis and blood pressure regulation. and Numerous physiological processes, such as blood pressure regulation, stress hormone response, fluid homeostasis, and cardiac contractility modulation, have been related to the apelin/APJ system (also known as an apelinergic system) (Reaux *et al.* 2001, Tatemoto *et al.* 2001, O'Carroll *et al.* 2003). Heart failure (Földes *et al.* 2003), obesity (Boucher *et al.* 2005), diabetes (Li *et al.*

2006), and cancer (Wang *et al.* 2008) are only a few of the pathological situations in which apolin plays a role.

2.18 Apelin in Cardiovascular Diseases

In a healthy heart, the cardiac myocytes express both sapelin and APJ. Studies conducted in a laboratory setting show that apolin improves cardiac output and has an inotropic impact (Szokodi *et al.* 2002). It also produces vasodilation in the arteries, which helps lower blood pressure (Japp *et al.* 2008). The first association between apelin and cardiovascular disease was discovered in 2003. In failing human hearts, increased levels of apelin mRNA expression were found compared to normal tissue, suggesting a potential involvement for apelin in the pathophysiology of heart failure (Földes *et al.* 2003). When administered to patients with heart failure, apolo raises cardiac output, blood pressure, and peripheral vascular resistance (Japp *et al.* 2010). Furthermore, it has been shown that [Pyr1]apelin-13 injection has a long-lasting cardioprotective effect against myocardial infarction in a rat model by decreasing infarct size, increasing heart rate, and increasing blood nitric oxide level over the course of several days. Additional investigation by (Azizi *et al.* 2013, Li *et al.* 2012). Reducing blood supply to the heart muscle causes tissue damage, a condition known as postmyocardial infarction (PMI) or heart attack. Animal studies using PMI to treat heart failure showed significant improvement in cardiac function and increased expression of SDF-1 and CXCR4, suggesting that these molecules may be useful therapeutic targets. This peptide may reduce cardiac hypertrophy and oxidative stress by limiting ROS formation (Foussal *et al.* 2010). A decrease in the prosurvival pathway mediated by phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) certainly contributed to the worsening of myocardial infarction-related mortality, infarct size, and inflammation in apelin-knockout rats (Wang *et al.* 2013). High blood pressure, or hypertension, is a known risk factor for cardiovascular illness. Increased risk of cardiovascular events including heart attack, stroke, vascular dementia, and renal failure have all been associated to long-term hypertension (Alam *et al.* 2017). Similar to newly diagnosed hypertension people, hypertensive rats showed decreased expression of both apelin and APJ mRNA (Akcilar *et al.* 2013, Sonmez *et al.* 2010). ApoE and apelin, two proteins, have been linked to blood pressure control. Left ventricular myocytes derived from hypertensive rats with heart failure showed decreased protein expression of this receptor. Treatment of hypertensive rats with apelin resulted in a decrease in left ventricular systolic pressure, while exogenous treatment with [Pyr1]-apelin-13 resulted in a substantial increase in APJ levels. Several lines of evidence indicate that nitric oxide synthase (eNOS) is the biological mechanism through which apelin and the APJ receptor cause hypotension (Ishida *et al.* 2004, Pang *et al.* 2014).

The significance of apolin as a biomarker for the detection of cardiovascular disease has been shown in a large number of clinical studies. Patients with coronary artery disease had reduced serum apelin-12 levels, according to a research by (Kadoglou *et al.* 2010). Systolic left ventricular failure patients had reduced concentrations of apelin-12 in both the blood and the heart muscle (Ye *et al.* 2015). Apolin-12 had a secretion pattern unique from the internal control brain natriuretic peptide, which is only produced in the heart. This pattern was seen in the coronary sinus, the aorta, and the renal vein (Chandrasekaran *et al.* 2010). This study confirmed that left ventricular pressure overload alters circulating apelin-12 transcardiac arteriovenous gradients. Left ventricular hypertrophy models were used in both humans and animals to test apolin36 levels (Helske *et al.* 2010). Although plasma apelin levels were greater in both groups, there was no increase in apelin expression in the heart muscle. A biomarker of left ventricular hypertrophy, plasma apelin levels have showed potential in human and animal investigations (Falco-Pires *et al.* 2010). An analysis of the available literature suggests that serum apelin (in any of its forms) may serve as a valuable athero-protective marker in preventing the development of coronary artery disease (Chen *et al.* 2017b).

These findings indicate that apelin has the potential to boost cardiac output and protect the heart from the damaging effects of myocardial infarction and oxidative stress. In cases of heart hypertrophy, the apelinergic system may serve as a pressure sensor in addition to regulating blood pressure. Apolin's hypotensive effects might originate in the Akt/eNOS pathway. Recent publications have provided in-depth analysis of the apelin/APJ system's function in cardiac hypertrophy (Lu *et al.* 2017).

2.19 Apelin and Cardiac Atherosclerotic Diseases

Myocardial infarction (MI) and ischemic heart disease are two examples of cardiac atherosclerotic disorders from which patients might benefit greatly from angiogenesis in order to make a full recovery. Animal models of ischemic heart disease and acute myocardial infarction have shown that apolo stimulates angiogenesis (Mao *et al.* 2011). Patients with MI had lower apelin levels, which was linked to poor myocardial angiogenesis (Li *et al.* 2010). With its paracrine function, apelin induced neovascularization in the peri-infarct region after being injected into the ischemic myocardium (Tempel *et al.* 2012). 14 days after myocardial infarction, apolin-13 has been demonstrated to enhance cardiac function, reduce cardiac hypertrophy, minimize cardiac fibrosis, and increase angiogenesis (Li *et al.* 2008). The homing of vascular progenitor cells and the production of SDF-1a/CXCR-4 are two potential pathways by which apelin-13 promotes angiogenesis after myocardial infarction (Wang *et al.* 2013). An further study validated apelin's angiogenesis action in the heart by treating mouse bone marrow cells with apelin before injecting them into the myocardium. There was an increase in myocardial angiogenesis and a lessening of heart fibrosis. Myocardial infarction is a frequent consequence of cardiac atherosclerotic illnesses, however because to Apolin's positive impact on myocardial angiogenesis, it may be used as a myocardial protective factor following infarction. Affirmation of apelin's beneficial benefits requires further extensive clinical studies (Kidoya *et al.* 2010).

2.20 Apelin and Hypertension

Arterial stiffness and endothelial dysfunction are linked to hypertension. Changes in artery structure and function are a hallmark of aging, and this is true even in otherwise healthy young individuals (Azizi *et al.* 2013). Age-related endothelial dysfunction is the primary cause of arterial stiffening as we become older (Li *et al.* 2013b). Blood channel dilation need nitric oxide (NO) (Laurent *et al.* 2001). Age-related reductions in eNOS mRNA and protein expression in the arteries have been linked to arterial stiffness and hypertension (Novella *et al.* 2013). Animal models of hypertension and normotension have shown that apolonius dramatically lowers blood pressure (Katugampola *et al.*

2001). Treatment with apelin causes nitric oxide (NO)-mediated arterial dilatation while having no effect on peripheral venous tonus (Quazi *et al.* 2009). The vasodilatory effects of apolin were decreased by a NOS inhibitor, indicating a potential function for nitric oxide (Szokodi *et al.* 2002). APJ-deficient animals did not exhibit the apelin-induced antihypertensive effects or the activation of eNOS in endothelial cells (Zhang *et al.* 2006). Endothelial dysfunction and arterial stiffness may be associated with age-related decreases in NO expression and plasma apelin levels. In addition, the antihypertensive activity of apelin is thought to be endothelium-dependent since its concentration-dependent vasodilatation impact was normal in mammary arteries with intact endothelium but reduced after endothelial ablation (Maguire *et al.* 2009).

2.21 Apelin and Atrial Fibrillation (AF)

Apolin is highly expressed in human atrial myocardium that is otherwise normal (Miettinen *et al.* 2007). Plasma apelin levels were significantly lower in individuals with atrial fibrillation compared to sinus rhythm controls (Francia *et al.* 2007). The circulating apelin level was shown to increase if patients maintained sinus rhythm (Ellinor *et al.* 2006). The atrial systolic function declines and the atrial tissue remodels as a result of atrial fibrillation. Patients with atrial fibrillation may have decreased atrial apelin synthesis due to elevated diastolic filling pressures. As a consequence of apelin's effect on ionic currents, the atrial action potential duration is reduced (Cheng *et al.* 2013). In the older population, atrial fibrillation is a major killer. In AF patients, the quantity of apelin may represent atrial systolic performance, according to previous studies. More research is needed to determine if apelin can accurately anticipate the severity of atrial fibrillation and whether or not sinus rhythm can be maintained.

2.22 Apelin and Other Diseases

2.22.1 Role of apelin in different types of cancer

Tumorigenesis, cancer cell migration, angiogenesis, resistance to apoptosis, and cancer's ability to metastasize might all be influenced by apelin. Several kinds of cancer cells may benefit from the proliferation and survival signals sent by various apelin peptides. Some instances include cancers of the prostate (Hoffmann *et al.* 2017).

Metastasis is only correlated with cancer cell migration. Apolin-13 induced lymph node metastasis of melanoma cells producing overexpressed apelin in mice (Berta *et al.* 2014). Epithelial-mesenchymal transition (EMT) has been linked to the first step of metastasis in cancer cells. There is still debate about whether or not the apelinergic system plays a part in EMT. Apolin-13 suppressed TGF-induced EMT in primary human renal proximal tubular epithelial cells. Increasing cell movement and spreading (metastasis) has been attributed to apelin. Because of Smad-2/3 and phospholipase C, this effect was suppressive (Wang *et al.* 2017).

The hypoxic environment inside a tumor is a major pathogenic component that favors an angiogenic shift. Tumor and host cells, such as macrophages, may also disturb the vascular network via the production of pro-angiogenic substances. Al-Abd *et al.* (2017) found that these pro-angiogenic molecules may function as a chemotactic signal, causing endothelial cells to migrate and proliferate inside tumor tissue and subsequently leading to the development of new vascular networks. Activation of PI3K, eNOS, and FAK phosphorylation have all been linked to this mechanism (Lamalice *et al.* 2007). Angiogenesis in tumor development may include the apelinergic system.

Tumor development was sped up in vivo and microvessel density (MVD) was enhanced when apelin-overexpressing NSCLC cells were implanted into mice (Berta *et al.* 2010). Hepatocellular carcinoma (HCC) patients tended to have greater MVD than healthy controls. In the same model, we also observed an upregulation of the transcript levels of the proangiogenic proteins apelin and angiopoietins 1 and 2. It has been shown that the apelinergic system promotes tumor development in a mouse model of hepatocellular carcinoma (HCC) by using an APJ antagonist, F13A, to suppress tumor growth (Muto *et al.* 2014). Upregulation of the APLN gene has also been linked to cancer cell metastasis, invasion, and angiogenesis in hepatocellular cell lines (Lin and Chuang, 2013).

Serum and tissue apelin levels in gastroesophageal cell carcinoma are much greater than in healthy samples, according to research by (Diakowska *et al.* 2014). Clinical features and prognosis were shown to be significantly correlated with apelin expression in gastric cancer (GC) tissue. Patients who expressed more apelin in their tumors fared worse than those whose tumors expressed less apelin. Apelin improved GC cells' capacity to migrate and invade in an in vitro investigation. Matrix metalloproteinase-1 (MMP-1), matrix metalloproteinase-9 (MMP-9), interleukin-1 (IL-1), and interleukin-6 (IL-6) are all involved in tumor invasion and metastasis, and their expression was all enhanced by apelin stimulation. The fact that siRNA-mediated downregulation of APLNR inhibited gastric cancer cell proliferation, migration, and invasion hints to a function for the apelinergic system in GC development (Feng *et al.* 2016).

In patients with GC, tissue expression of the APJ protein was shown to be considerably greater in those who had a poor response to chemoradiotherapy and endostar, a modified recombinant human endostatin (Hao *et al.* 2017).

In prostate cancer, apelin also has mitogenic activity. Prostate cancer cell lines treated with apelin-13 showed enhanced proliferation (Tekin *et al.* 2014). The finding that the tumor-suppressor miR224 is complementary to the 3′UTR of the APLN mRNA provides more support for the idea that apelin is a direct target of miR-244. The anti-migration and anti-invasion effects of miR-224 in prostate cancer cells were lost when APLN was knocked down. It has also been observed that miR-244 has an inverse relationship with APLN expression levels. Tumor development aggressiveness in prostate cancer patients was connected with downregulation of miR-244 and overexpression of APLN (Wan *et al.* 2015).

Endometrial cancer patients had elevated serum apelin-36 levels, which correlated with both BMI and fasting insulin. However, Altinkaya *et al.* (2015) found no correlation between apelin-36 expression and tumor histology subtype or size.

An higher blood apelin level was linked to an increased risk of cancer development and mortality in individuals with hyponatremia, a chronic kidney illness. Based on these findings, apelin may be of benefit in the prognosis of this particular kind of cancer (Lacquaniti *et al.* 2015).

Expression of apelin-36 was low in tissue derived from oral squamous cell carcinomas. Furthermore, there was no association between apelin expression and patients' overall survival. In vitro, apelin-13 stimulation of oral cancer cells led to elevated ERK kinase phosphorylation. Also, oral cancer cells were shown to proliferate and migrate when exposed to apelin (Heo *et al.* 2012).

Overall, many different cancers share the presence of apelin and its receptor. The mRNA or peptide/protein levels of apelin/APJ are often higher than in healthy controls. It's possible that the apelinergic system contributes to cancer development as well. Several studies suggest that the apelin/APJ system has a function in regulating cancer cell proliferation, migration, and invasion. In addition, apelin prevents apoptosis during tumor growth and participates in pathological angiogenesis.

2.22.2 Apelin in obesity

Physiologically active substances termed adipokines are secreted by adipocytes, the major cell type in adipose tissue (Tapanetal.,2010).The stromal-vascular component of this dynamic tissue consists of blood cells, pericytes, endothelial cells, and adipose progenitors in addition to mature adipocytes. Adipose tissue does more than only store fat, it also secretes biologically active substances that help keep the body's metabolism in check (Coelho *et al.* 2013). Apelin, a peptide found in adipose tissue, may have an influence on diseases associated with obesity. Its function is still mysterious, however, and the results of experiments have been contradictory. A decrease in plasma apelin-12 levels was seen in young individuals with obesity, which may be linked to insulin resistance (Tapan *et al.* 2010). The plasma concentration was also shown to be significantly higher in the obese female children compared to the normal weight youngsters in another investigation (Ba *et al.* 2014). Blood levels of the hormone apelin-12 are also shown to be higher in persons who are overweight or who have insulin resistance as a result of their weight (Krist *et al.* 2013).

Obese adults who followed a hypocaloric diet had a significant decrease in plasma apelin (all forms). Alterations in plasma apelin levels caused by food were linked to lower levels of insulin and tumor necrosis factor alpha. In contrast, a low-calorie meal decreased expression of apelin and APJ (Castan-Laurell *et al.* 2008).

Inhibiting lipolysis in adipocytes is another role of apelin. PKA-mediated phosphorylation of hormone-sensitive lipase at Ser-563 is blocked by [Pyr1], whereas AMPK-mediated phosphorylation is stimulated.Injecting -apelin-13 into cultured adipocytes reduced the amount of lipolysis caused by isoproterenol (Yue *et al.* 2011).

The differentiation of preadipocytes into mature adipocytes was shown to be suppressed by [Pyr1]-apelin-13 via activation of the AMPK and MAPK/ERK pathways. By decreasing the number of mature adipocytes and increasing the size of intracellular lipid droplets, exogenous apelin has been demonstrated to impede lypolysis (Than *et al.* 2012).

The connection between angiogenesis and the growth of adipose tissue is noteworthy. Obesity is linked to an increase in adipocytes, which may have detrimental effects on lymphatic channel structure and function. Lymphatic and vascular walls are fortified by apolin signaling. Apelin inhibited fat accumulation by counteracting the effects of dietary fatty acids on the permeability of lymphatic and circulatory vessels (Sawane *et al.*., 2013).

2.22.3 Apelin in a type 2 diabetes

The expression of apelin in the pancreatic islets is controlled by glucocorticoids but not glucose (Ringström *et al.* 2010). All mammals, including pigs, cats, mice, and rats, have this trait. Apolin boosts both glucose uptake and insulin sensitivity, making it easier for the body to metabolize fuel (Bertrand *et al.* 2015).

Patients with higher apelin-12 concertation were more likely to develop diabetes, suggesting that monitoring plasma apelin levels may be used as a predictor of the beginning of type 2 diabetes (Zheng *et al.* 2016).

In insulin-resistant adipocytes, translocation of glucose transporter type 4 (GLUT-4) was triggered by [Pyr1]-apelin-13 in an Akt/PI3K-dependent manner, resulting in enhanced inflammatory responses and glucose uptake (Zhu *et al.* 2011). It's exciting to think that apelin may have a role in controlling blood sugar by activating AMPK and lowering cAMP (Alipour *et al.* 2017).

3. MATERIALS AND METHODS

3.1 Material

3.1.1 Study design

Where this case study was conduction from September 2022until April 2023 at Çankırı Karatekin Üniversitesi/ Department of Biochemistry on 145 people The first group included healthy people, meaning the control group, which consists of 67 healthy people, and the second group included 78 people with heart disease(CAD), where the control group was compared with the patient group through a study measuring the concentration of Apelin with some biochemical variables on the disease .Information was collected from patients at Al-Salam hospital and Cardiac Surgery Center in Mosul and during their interview information was taken about their demographic characteristics, age, gender and other information.

3.1.2 Blood collection

Careful preparation of the skin at the venipuncture site included the use of a bacterial disinfectant (70 percent alcohol) and the subsequent drying of the disinfectant. Each patient had around 3 milliliters of blood taken with a sterile syringe. The withdrawn blood then poured in a Gel tube where allowed for clotting in 15 minutes then allowed rotating in the centrifuge at a rate 15000 R.P.M for separating it into serum. After that, the serum is collected into another test tube and allowed to refrigerate until further use.

3.1.3 Grouping of patient and control

1-Group -1 (control) 67 blood sample will be collected from normal individual and matched to patients.

2-Group-2 (patient with heart disease)78 blood sample collected from patient will heart diseases.

3.1.4 Chemical, equipment and instruments

The following equipment were used throughout the study as shown in the Table 3.1.

Table 3.1 Equipment used and their companies

No.	Equipment	Company	Country
1	Cobas c111	Roche Diagnostics GmbH	Germany
2	Centrifuge	Hettich	Germany
3	Spectrophotometer	Cecil instrument	Uk
4	Incobater	Gottingen	Germany
5	Alcohol 70%	Olive pond	Iraq
6	Gel tube	Alfa-Med	China
7	Syringes	Green	China
8	Ependoef tube	Numedico	USA
9	Ependorf rack	Numedico	UAS
10	ELISA	Biokit ELx	USA
11	Micropipettes	Hirschmann	Germany
12	Tips	Afco	Jorden

3.1.5 Reagents and kits

Chemicals and starter kits, tabulated Table 3.2.

Table 3.2 The chemicals and kits

No	Reagent and kits	Lot No.	Company	Country
1	Apelin	ELK8309	Elk	USA
2	Serum creatinine	REF05401755 190	Roche	Germany
3	Bl.urea	REF04657616 190	Roche	Germany
4	S.ALT	REF04718569 190	Roche	Germany
5	S.cho.	REF04718917 190	Roche	Germany
6	T.G	REF04657594 190	Roche	Germany
7	HDL-C	REF07528604 190	Roche	Germany
8	Catalase	ELK1318	ELk	USA
9	S.Ca	REF05061504 190	Roche	Germany
10	S.sodium	REF1103110015	ELYTE	INDIA
11	Uric acid	REF(240)04657608190	Roche	Germany
12	S.Alb.	REF80002	BioLABO	France

3.2 Methods

3.2.1 Estimation of (Apelin) by ELISA

Test principle: This kit use the sandwich enzyme immunoassay technique for testing. An Apelin(APLN) antibody-coated microtiter plate is included in this set. A biotin-conjugated antibody specific for Apelin(APLN) is then added to the microtiter plate wells that have previously contained standards or samples. The next step is to add Avidin-Horseradish peroxidase (HRP) to each microplate well. After adding the TMB substrate solution, the only wells that will continue to light are those that contain Apelin(APLN). The addition of biotin to an antibody and enzyme to Avidin will result in a change in color. The enzyme-substrate reaction is inhibited with sulfuric acid solution, and the ensuing color change is measured spectrophotometrically at 450 nm 10 nm. The amount of Agplin(APLN) in a sample may be determined by plotting its optical density (OD) against a standard curve.

3.2.2 Measurement of creatinine

Principle: This kinetic colorimetric analysis relies on the Jaffé method. In alkaline solution, creatinine and picrate form a yellow-red combination. The rate at which dye is produced is dependent on the amount of creatinine present in the sample. A method known as "rate-blanking" is employed to lessen the influence of bilirubin on the assay. To correct for the non-specific reaction caused by pseudo-creatinine chromogens in serum and plasma, such as proteins and ketones, results are lowered by -18 µmol/L.

3.2.3 Measurement of urea

Principle: Glutamate dehydrogenase and urease kinetic assay Hydrolysis of urea by urease yields ammonium and carbonate Equation (3.1).

$$Urea\ +\ 2\,H2O \xrightarrow{Urease} 2\,NH4* \ + CO32 \tag{3.1}$$

In the second step, ammonium, glutamate dehydrogenase (GLDH), and the cofactor NADH convert 2-oxoglutarate to L-glutamate. It takes two moles of NADH to produce one mole of NAD during urea hydrolysis Equation (3.2).

$$NH4^{+} \;+\; 2-oxoglutarate \;+\; NADH \xrightarrow{GLDH} L-glutamate \;+\; NAD +\; H2O \tag{3.2}$$

The rate of NADH depletion is a photometrically measurable function of the urea content in the material.

3.2.4 Measurement of ALT

Principle: This assay was developed in accordance with IFCC guidelines, but with enhanced throughput and reliability. The conversion of L-alanine to 2-oxoglutarate is catalyzed by ALT. In a process catalyzed by lactate dehydrogenase (LDH), pyruvate is reduced by NADH to yield L-lactate and NAD. The amino transfer process requires pyridoxal phosphate as a cofactor. It guarantees complete enzyme activity Equation (3.3) and Equation (3.4).

$$L-alanine \;+\; 2-oxoglutarate \xrightarrow{ALT} pyruvate \;+\; L-glutamate \tag{3.3}$$

$$Pyruvate \;+\; NADH \;+\; H \;\rightarrow\; L-lactate \;\;+\;\; NAD \tag{3.4}$$

NADH oxidation rate is proportional to ALT activity as a catalytic enzyme. The decline in absorbance is the indicator.

3.2.5 Measurement of sodium

Principle: A triple salt of sodium, magnesium, and uranium acetate is precipitated. In an acidic media, excess uranyl ions react with ferrocyanide to produce a brownish hue.

The sodium content in the sample has a negative correlation with the intensity of the resulting color Equation (3.5) and Equation (3.6).

$$Uranyl\ ions\ +\ Mg\ ions\ +\ Na' \rightarrow\ UranylMgNa\ precipitate \qquad (3.5)$$

$$Free\ Uranylions\ +\ K4\ Fe(CN)6\ \ \rightarrow Brown\ coloured\ complex \qquad (3.6)$$

3.2.6 Measurement of calcium

Principle: When 5-nitro-5methyl-BAPTA (NM-BAPTA) is exposed to an alkaline environment, calcium ions react with it to produce a complex. In a second stage, this compound reacts with EDTA Equation (3.7) and Equation (3.8).

$$Ca\ \ + NM - BATPA\ \ \xrightarrow{alkaline\ pH} calcium - NM - BATPA \qquad (3.7)$$

$$Calcium - NM - BATPA\ complex\ \ +\ EDTA\ \rightarrow\ NM - BATPA\ +$$
$$calcium\ EDTA\ complex \qquad (3.8)$$

The photometric measurement of the change in absorbance is directly proportional to the calcium content.

3.2.7 Measurement of albumin

Principle: Albumin content is related to the absorbance at 630nm (620-640), which is produced when bromocresol green binds to albumin in a 4.2 pH buffered solution.

3.2.8 Measurement of cholesterol

Principle : Enzymatic, colorimetric method.

Cholesterol esterase acts on cholesterol esters, breaking them apart into free cholesterol and fatty acids. Once oxidized, cholesterol is converted to cholest-4-en-3-one and hydrogen peroxide by the catalytic action of cholesterol oxidase. A red quinone-imine dye is produced by the oxidativecoupling of phenol and 4-aminoantipyrine in the presence of peroxidase Equation (3.9), Equation (3.10) and Equation (3.11).

$$Cholesterol\,esters\ +\ H2O\ \xrightarrow{CE}\ cholesterol\ +\ RCOOH \quad (3.9)$$

$$Cholesterol\ +\ O2\ \xrightarrow{CHOD}\ cholest-4-en-3-one\ +\ H2O2 \quad (3.10)$$

$$2\,H2O2\ +\ 4-AAP\ +\ phenol\ \xrightarrow{POD}\ quinone-imine\,dye\ +\ 4\,H2O \quad (3.11)$$

The cholesterol content is directly correlated with the dye's intensity. The rise in absorbance is the indicator.

3.2.9 Measurement of HDL- cholesterol

principle:Homogeneous enzymatic colorimetric test.

When non-HDL lipoproteins like LDL, VLDL, and chylomicrons are combined with polyanions and a detergent, they produce a water-soluble complex. When bound together, CHER and CHOD are unable to catalyze the breakdown of low-density lipoproteins. Only HDL-particles may combine with CHER and CHOD. HDL-cholesterol levels are analyzed by the enzymes CHER and CHOD. Cholesterol esters may be broken down into free cholesterol and fatty acids by the enzyme CHER Equation (3.12).

$$HDL-cholesterol\,esters\ +\ H2O\ \xrightarrow{CHER}\ HDL-cholesterol\ +\ RCOOH \quad (3.12)$$

In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to Δ4-cholestenone and hydrogen peroxide Equation (3.13).

$$HDL - cholesterol \ + \ O2 \ \xrightarrow{CHOD} \ \Delta 4 - cholestenone \ + \ H2O2 \tag{3.13}$$

The color is produced when hydrogen peroxide combines with 4-amino-antipyrine and EMSE3) in the presence of peroxidase. This dye's photometrically-measured color intensity is directly related to the cholesterol content Equation (3.14).

$$2\,H2O2 \ + \ 4 - amino - antipyrine \ + \ EMSE \ + \ H * \ + H2O$$
$$\xrightarrow{Peroxidase} \ colored\,pigment \ + 5H2O \tag{3.14}$$

3.2.10 Measurement of triglycerides

Principle: Enzymatic colorimetric test Equation (3.15), Eqution (3.16), Equation (3.17) and Equation (3.18).

$$Triglycerides \ + 3H2O2 \ \xrightarrow{LPL} \ glycerol \ + \ 3\,RCOOH \tag{3.15}$$

$$Glycerol \ + ATP \ \xrightarrow{MG\ ,Gk} \ glycerol - 3 - phosphate \ + \ ADP \tag{3.16}$$

$$glycerol - 3 - phosphate \ + O2 \ \xrightarrow{GPO} \ dihydroxyaceton \ phosphate \ + H2O \tag{3.17}$$

$$H2O \ + 4 - aminophenazone \ + 4 - chlorophenol \ \xrightarrow{peroxidase} 4 - (p -$$
$$benzoquinonemonoimino\,)\_phenazone \ + H2O \ + HCl \tag{3.18}$$

3.2.11 Measurement of uric acid (UA)

Principle: When uric acid is converted to allantoin by uricase, hydrogen peroxide is generated, which reacts with 4-aminoantipyrine and the dye in the presence of peroxidase (POD), resulting in a hue that is proportional to the concentration of uric acid in the sample.

3.2.12 Measurement of catalase in serum

Principle: For vanadium (V) to be reduced to vanadium (II), an acidic environment including the interaction of ammonium metavanadate with H2O2 is required. Depending on the redox circumstances, hydrogen peroxide (H2O2) may either be a powerful oxidant or a reluctant oxidant. Thus, the Peroxovanadium complex, with a maximum absorbance at 452 nm, is formed when vanadium (V) is reduced.

3.3 Statistical Analysis

Statistical analysis is used to compare the significant differences between the averages of the two group and for all parameter ,the t-test was used ,and pearson correlation analysis was employed to find the correlation relationship among the varibles ,using the SPSS-23 progra.

4. RESULTS AND DISCUSSION

Indicators of health in both patients and healthy controls Table 4.1.

Table 4.1 The characteristics of patient and healthy people(control)

Characteristics	Control	Patient
Age	47-58 years	56-69 years
Gender	Male	Male
Weight	67-85kg	65-80kg
Smoking	69%	73%

4.1 Result

Through a study conducted on 78 people with heart disease aged between 56 and 69 years and compared with 67 healthy people with heart disease who were aged between 47 and 58 years, it was found through this study that there was a significant decrease ($p \leq 0.01$)in the concentration of Apelin for a group of patients as shown in Table 4.2 and Figure 4.1 as well as a significant increase ($p \leq 0.01$) in both levels of total cholesterol,and triglycerides as shown in the Table 4.3 and Figure 4.2 as well as there is a significant increase ($p \leq 0.01$)in both serum creatinine and blood urea levels, as well as in uric acid, as in Table 4.4 and Figure 4.3. It was also noted that there is a significant decrease ($p \leq 0.01$) in the levels of HDL high-density lipids, as in Table 4.5 and Figure 4.4, as well as a significant decrease ($p \leq 0.05$) in the concentrations of antioxidants Catalase (CAT) as in Figure 4.4 through this study, it was also found that there are no significant differences between the two groups of patients and statistical subjects, where the levels of serum albumin, Alanine aminotransferase , serum calcium and serum sodium in patients and controls were close to each other as shown in Table 4.6 and Figure 4.5.

Table 4.2 Shows the graph of the apelin level

Parameters	Control	Patient
Apelin (pg/ml)	784 ± 3.07	667 ± 1.91

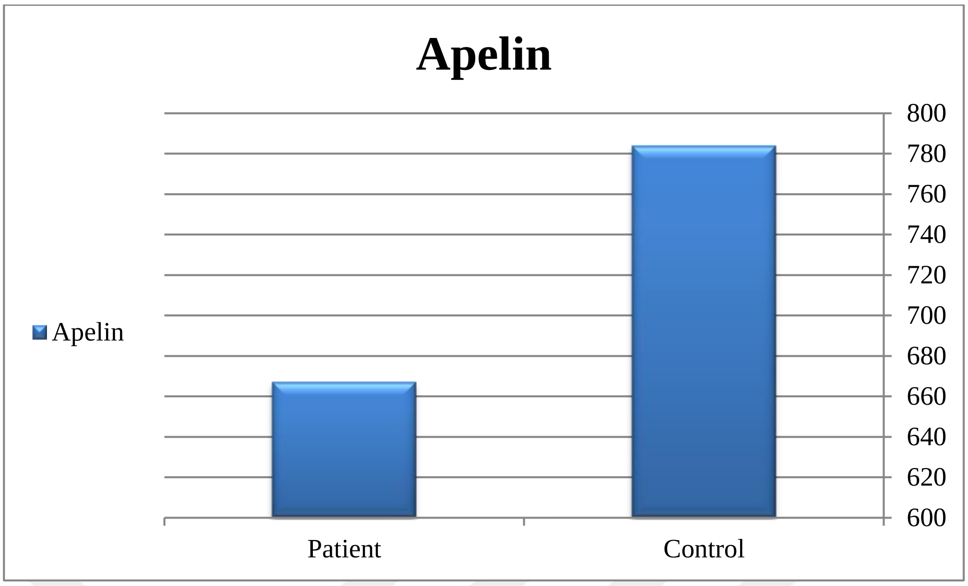

Figure 4.1 Shows the graph of the apelin level

Table 4.3 Shows the graph of total cholesterol and triglycerides level

Parameters	Control	Patient
Cholesterol (mmol/l)	3.66 ± 0.71	5.97 ± 0.29
Triglycerides (mmol/l)	1.69 ± 0.33	5.13 ± 0.34

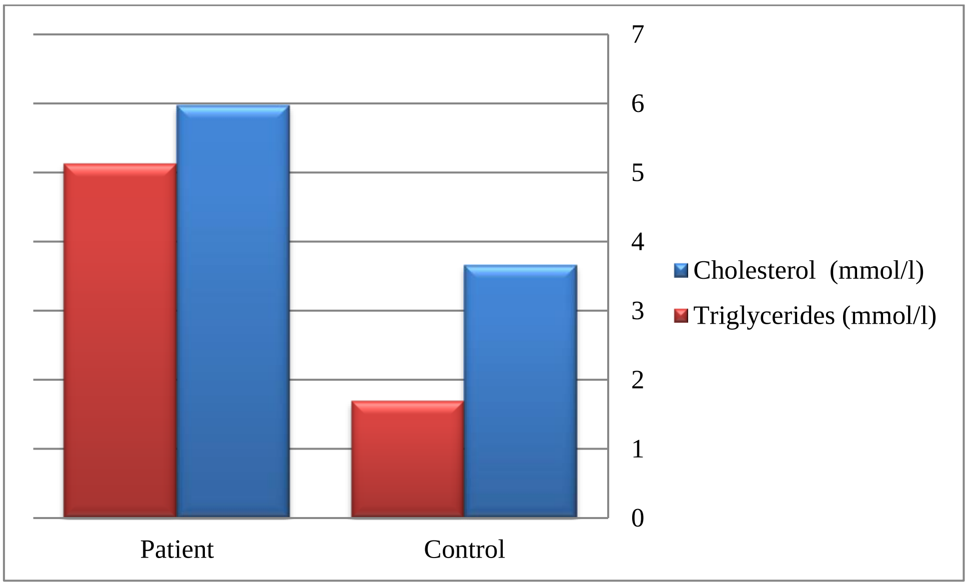

Figure 4.2 Shows the graph of total cholesterol and triglycerides level

Table 4.4 Shows the graph of the renal function level

Parameters	Control	Patient
Urea (mg/dl)	26.7 ± 2.80	70.3 ± 3.10
Creatinine (mmol/l)	83.3 ± 2.79	142 ± 2.47
Uric acid (mmol/l)	369 ± 4.02	467 ± 4.5

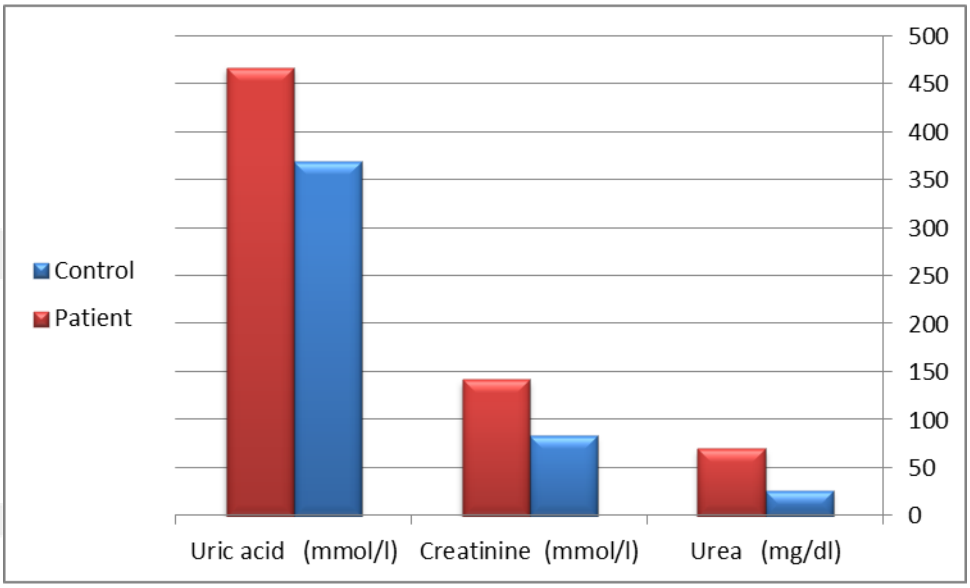

Figure 4.3 Shows the graph of the renal function level

Table 4.5 Shows the graph of HDL-C and CAT enzyme level

Parameters	Control	Patient
HDL (mmol/l)	1.37 ± 0.21	0.87 ± 0.09
Catalase (ku/l)	12.4 ± 0.21	7.52 ± 0.18

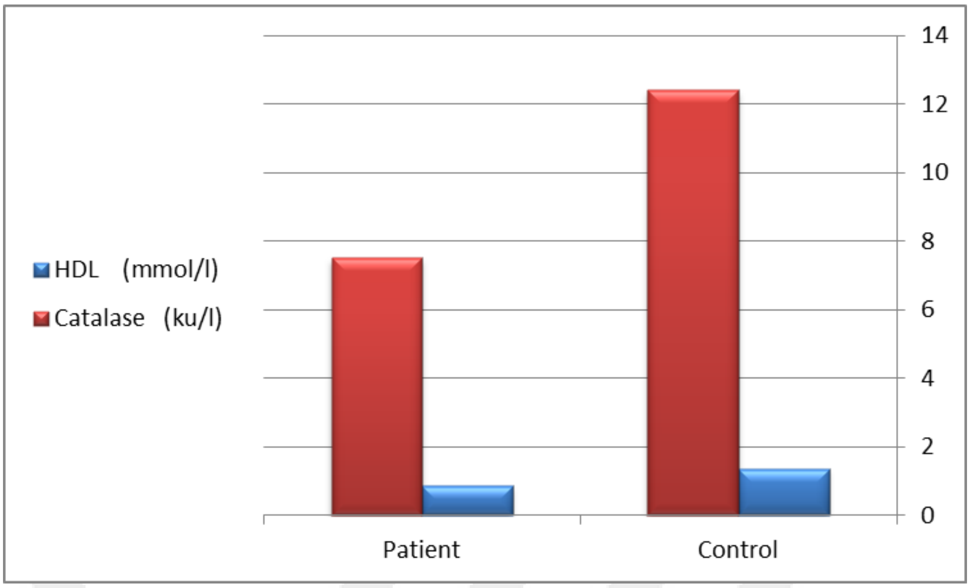

Figure 4.4 Shows the graph of HDL-C and CAT enzyme level

Table 4.6 Shows the graph of S.Alb.,S.Ca,S.Na,S.ALT level

Parameters	Control	Patient
Na (mmol/l)	138 ± 2.03	141 ± 4.56
Ca (mg/dl)	8.78 ± 0.06	8.85 ± 0.05
ALT (U/L	36.7 ± 4.69	38.3 ± 2.13
Albumin (g/l)	38.3 ± 2.38	44.6 ± 2.75

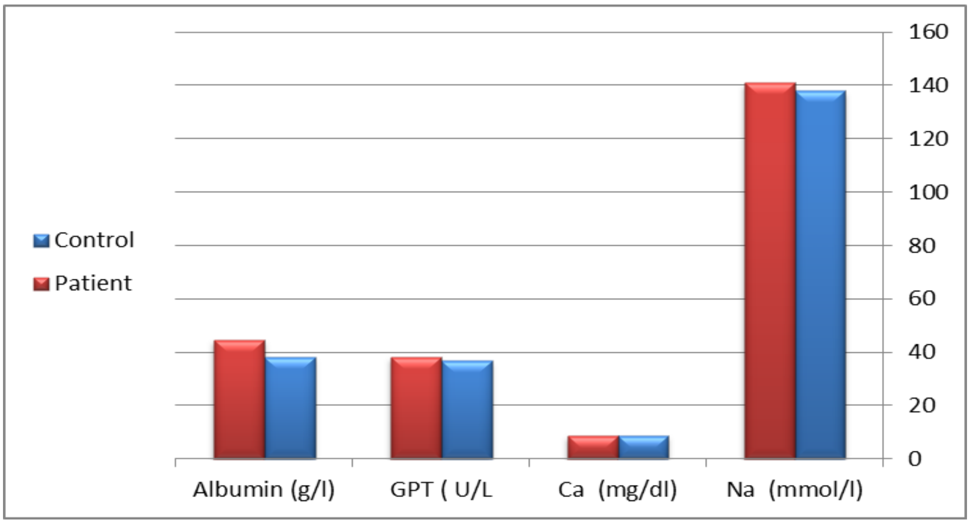

Figure 4.5 Shows the graph of S.Alb.,S.Ca,S.Na,S.ALT level

4.2 Discussion

Results showed a significant decrease ($p<0.01$) in the concentration of Aplin for the patient group, with the concentration of Aplin being lower in infected people compared to the group of healthy people, and these findings were consistent with the results of both previous and current studies on the characteristics of the concentration of Aplin serum in patients with chronic heart disease across a wide range of severe diseases (Zhuo 2013, Zhu *et al.* 2013).

The reason for the decrease in the concentration of Aplin in patients is due to damage to the endothelial cells located in the blood vessels, where the production of nitric oxide decreases, which prevents the deposition of harmful fats in the lining of blood vessels, and therefore the blood vessels expand and blood pressure decreases.

Furthermore, findings agree with (Foldes *et al.* 2003) in that patients with CHF owing to left ventricular systolic dysfunction had considerably lower plasma apelin concentrations compared to normal controls. Aplin has been demonstrated to have a beneficial inotropic effect in addition to not being a vasodilator (Szokodi *et al.* 2002).

The results of the study also showed that there is a significant increase of $p<0.01$ in both blood urea and serum creatinine and uric acid, if the relationship between both the level of Aplin and kidney function is inversely related, the higher the concentration of urea, creatinine and uric acid, the lower the concentration of Aplin and the opposite happens when the concentrations of Aplin decrease, where these results correspond to the results of Since a decrease in Aplin level is associated with an increase in the risk of heart disease, we have also come closer to the Association of Aplin levels with kidney function and cardiovascular risk factors .our results suggest that Aplin levels were inversely related to known cardiovascular risk factors that are directly related to marked kidney function. thus, it seems that the more Aplin improves kidney and cardiovascular function, the lower the risk.These data seem to be consistent with the previously reported study (El-Shehaby *et al.* 2010).

The result in this study indicates a significant increase in cholesterol concentrations $p<0.01$ compared to healthy people, atherosclerosis and cardiovascular diseases occur directly due to the accumulation of fat in macrophages, especially cholesterol and macrophages are inflammatory cells that promote atherosclerotic lesions and are the main component of sclerotic plaques (Duncan *et al.* 2019).

The reason for the formation of free radicals in atherosclerosis may be due to a decrease in the effectiveness of the enzyme peroxidase, which increases the formation of cholesterol carrier, which increases the formation of oxysterol in the blood, activation of the effectiveness of LDL particle receptors and its high level in the blood (Arnold *et al.* 2021).

It has also been observed that there is a significant increase $p<0.01$ in the level of TG triglycerides in the blood serum of patients compared to healthy people, the reason for this increase is that TG molecules are an energy for inflammation-causing receptors (prostoclandins and leukotrienes) with a decrease in the effectiveness of the lipoprotein enzyme responsible for the decrease of TG in very low density lipoprotein particles, the main carrier of TG (Ruban *et al.* 2020).

As a predictor of cardiovascular disease risk, high density lipoprotein (HDL) cholesterol (HDL_C) was found to be significantly lower in patients than in controls due to a combination of factors, including a decrease in the efficiency of the lipoprotein enzyme lipase HDL and inhibition of the enzyme lecithin cholesterol acyltransferase, which is necessary for HDL_C, and a decrease in the production of nascent or primary particles from cells of the small. As the progenitor and endothelial cells work to identify with the effective repair of the endothelium and the Prevention of endothelial dysfunction and the development of lesions resulting from atherosclerosis, vascular rupture and vasoconstriction because it is responsible for the transfer of excess cholesterol from the surrounding tissues to the liver, the imbalance of this process leads to the accumulation of cholesterol and the development of heart disease (Harvey *et al.* 2017).

The result in this study also showed a significant decrease in the effectiveness of the catalase enzyme in cardiac patients compared with the control group, if the enzyme is one of the enzymatic antioxidants and works to convert hydrogen peroxide into water and oxygen and produces atherosclerosis and heart disease large amounts of hydrogen peroxide and thus will lead to a high increase of oxidative damage and the production of free radicals on the walls of blood vessels such as peroxynitrite and thus the effectiveness of the enzyme decreases through the competition of the free radical to bind to the active site in the enzyme (Cangut *et al.* 2019).

In this study, there was a result of an insignificant increase in the effectiveness of some biochemical indicators, such as the effectiveness of the ALT enzyme, as well as in both sodium and calcium salts.

5. CONCLUSIONS AND RECOMMENDATION

Through this study conducted on a group of people who have problems with the heart and blood vessels, we conclude that Apelin peptide has an important role in the Prevention of heart disease, that is, the higher the level of Apelin in the blood reduces the risk of heart disease, as well as it has an important role in hypertension, obesity and cancer, as well as plays an important role in Type II diabetes, and we also note that antioxidants have a role in disease prevention by reducing free radicals and reducing hydrogen peroxide if it turns into water and oxygen. Due to the importance of Apelin peptide, the study recommends the following:

1-studying the effect of the concentration of Apelin on the nervous system and its role in the treatment of Disease Which infect this organ.

2-studying the effect of Apelin concentration on sex hormones and its role in the treatment of. Infertility in both sexes.

3-studying the effect of the concentration of Apelin on An cancer diseases and is it possible Use as an anti-cancer drug.

4-studying the effect of the Apelin concentration on the levels of the grouth hormone and its relationship to the two phenomena. Dwarfism and gigantism.

5-studying the impact of Apelin focus on allergic diseases and asthma and its relationship with improving Respiratory function.

6-studying the effect of Apelin concentration on the process of producing red blood cells and its habit With anemic diseases.

7-studying the effect of Apelin concentration on some A2 enzymes such as phosphate enzymes Basal and acidic liver function.

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