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Biochemical Assessment of Certain adipokines and their Association with Cardiac Arrhythmia

A Thesis

Submitted to the Council of the College of Medicine University of Kerbala in Partial Fulfillment the Requirement for the Master Degree in Clinical Biochemistry

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بسم الله الرحمن الرحيم

وَيَسْأَلُونَكَ عَنِ الرُّوحِ ۖ قُلِ الرُّوحُ مِنْ أَمْرِ رَبِّي
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صدق الله العلي العظيم

الاسراء ٨٥

Supervisor's Certification

We certify that this thesis M. Sc. entitled:

Biocheical Assessment of Certain Adiopokines and their Association with Cardiac Arrhythemia

Was presented by **(Ahmed Abdel Hameed Jawad)** and made under our supervision at the department of chemistry and biochemistry, college of medicine, university of kerbala, as a partial requirement for the degree of master in **Clinical Chemistry.**

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Dedication…

To who turned adversity into a grant…Allah (¤)

To the savior of the world's injustice…Imam Mehdi…

To the holy blood…The martyrs of Iraq…

To my angels in life…My parents…

To my second half and companion of my life… My Wife…

To the roses blooming in the hardest days…My sister …

To encouraged me and helped me the length of my life Scholastic…All teachers and my friends…

Dedicate this work with my love and gratitude…

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Summary

background: Among all types of CVDs, arrhythmias are most often responsible for sudden deaths and are indicative of other high-risk diseases. Arrhythmia is the collective term for a variety of conditions that involve a heart rhythm other than sinus rhythm. Adipokines are produced from adipose tissues, which are considered endocrine organs involved in cardiovascular function causing electrophysiological effects such as ionic profiles, contractility in the atrium, and change in morphology of action potential. Nesfatin-1 and chemerin are a newly discovered adipokine, related to the inflammatory process.

The present study aims to examine the diagnostic ability of adipocytokines (Nesfatin-1 and chemerin) and their ratio in SVT arrhythmia cases.

Material and methods: The study recruited 60 patients and 30 healthy controls. These patients were divided into two subgroups of SVT, certain etiology of SVT cases with clear history of one or more of the risk factors such as (lipid disorder, thyroid disorder, DM, hypertension), and uncertain etiology of SVT cases without any previous history. Serum Nesfatin-1 and chemerin were measured using the ELISA technique. Some related parameters were also determined and correlated with the level of these adipokines.

Results: The mean level of serum nesfatin-1 was significantly higher in normal subjects than in both SVT with risk factor & SVT without risk factor etiology groups.

Estimation plot of determination serum level of Chemerin indicted a massive increased level in SVT without risk factor patients compared the other groups.

Binary logistic regression was performed. It was found that chemerin in SVT with risk factor and SVT without risk factor etiology SVT patient (OR: 1.008 and 1.018;

95% CI: (0.998 - 1.018), (1.008 - 1.028) respectively, were independent risk factors. while Nesfitin (OR: 0.960 and 0.946, 95% CI: (0.929 - 0.992), (0.913 - 0.980) were independent protective factors for arrhythmia SVT patient. Results of the receiver operating curve (ROC) and AUC analysis for the optimal diagnostic points for predicting SVT without risk factor etiology of SVT cases was indicated that chemerin was demonstrated the most interesting prediction (sensitivity = 0.60.%, specificity = 0.89%) at a level = 733.55).

Conclusions: Nesfatin-1 and chemerin levels are affected by SVT arrhythmia disease when adjusted for other cofounders. The present results suggest that serum chemerin can be used as an inflammatory marker of SVT arrhythmia patients as it has good sensitivity and specificity.

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List of Abbreviations

ACE	Angiotensin-Converting Enzyme
AF	Atrial Fibrillation
AUC	Area Under Curve
BMI	Body Mass Index
CAD	Computer-Aided Design
CI	Confidence Interval
CIMT	Carotid Intima-Media Thickness
CKD	Chronic Kidney Disease
CVDs	Cardiovascular Diseases
D	Vitamin D3
DM	Diabetic Mellitus
EC	European Commission
ECG.	Electrocardiography
HDL	High Density Lipoprotein
LDL	Low Density Lipoprotein
LPL	Legends Pro League
LTRE	Long-Term Recording ECG
MRI	Magnetic Resonance Imaging
OR	Odd Ratio
ORS	Orthopaedic Research Societyhttps

ROC	Receiver Operator Characteristics
SVT	Supraventricular Tachycardia
TG	Triglyceride
VCAM	Vascular Cell Adhesion Molecule
VGCC	Vance-Granville Community College
VLDL	Very Low-Density Lipoprotein

Chapter one

Introduction
&
Review of Literature

1. Introduction:

1.1.The Heart

The heart is a muscular organ located in the central region of the thorax, in the middle and lower mediastinum, between the two lungs. The primary function of the heart is to pump a continuous flow of blood through the blood vessels to provide the body with oxygen and nutrients. The pump rate is determined by the pacemaking cells located at the sinoatrial node, that generate autonomously the initial impulse that travels at fast speeds across the cardiac conduction system. Therefore the electrical impulse spreads through the heart triggering and coordinating the contraction of the cardiac cells that pump blood to the body. The heart is a complex organ in which three main different physics interact: electrophysiology, mechanics, and hemodynamics (1).

The contraction and relaxation of the cardiac muscle follow a specific synchronized pattern between the atria and the ventricles. This rhythmic contraction and relaxation is preceded by electrical activity called the action potential that are represented in the depolarization and repolarization of the cardiac muscle, respectively. The special origin and sequence of the initiation and propagation of the action potential are essential for maintaining normal heart function. The action potential is created by ions fluxes across the plasma membrane of the cardiac muscle cells via specific channels, transporters, and other proteins. Normally, the action potential originates in the sinoatrial (SA) node known as “the pacemaker of the heart,” which propagates a specific sequence in the atria first and then in the ventricle through specialized conducting tissues (2) as shown in figure (1.1).

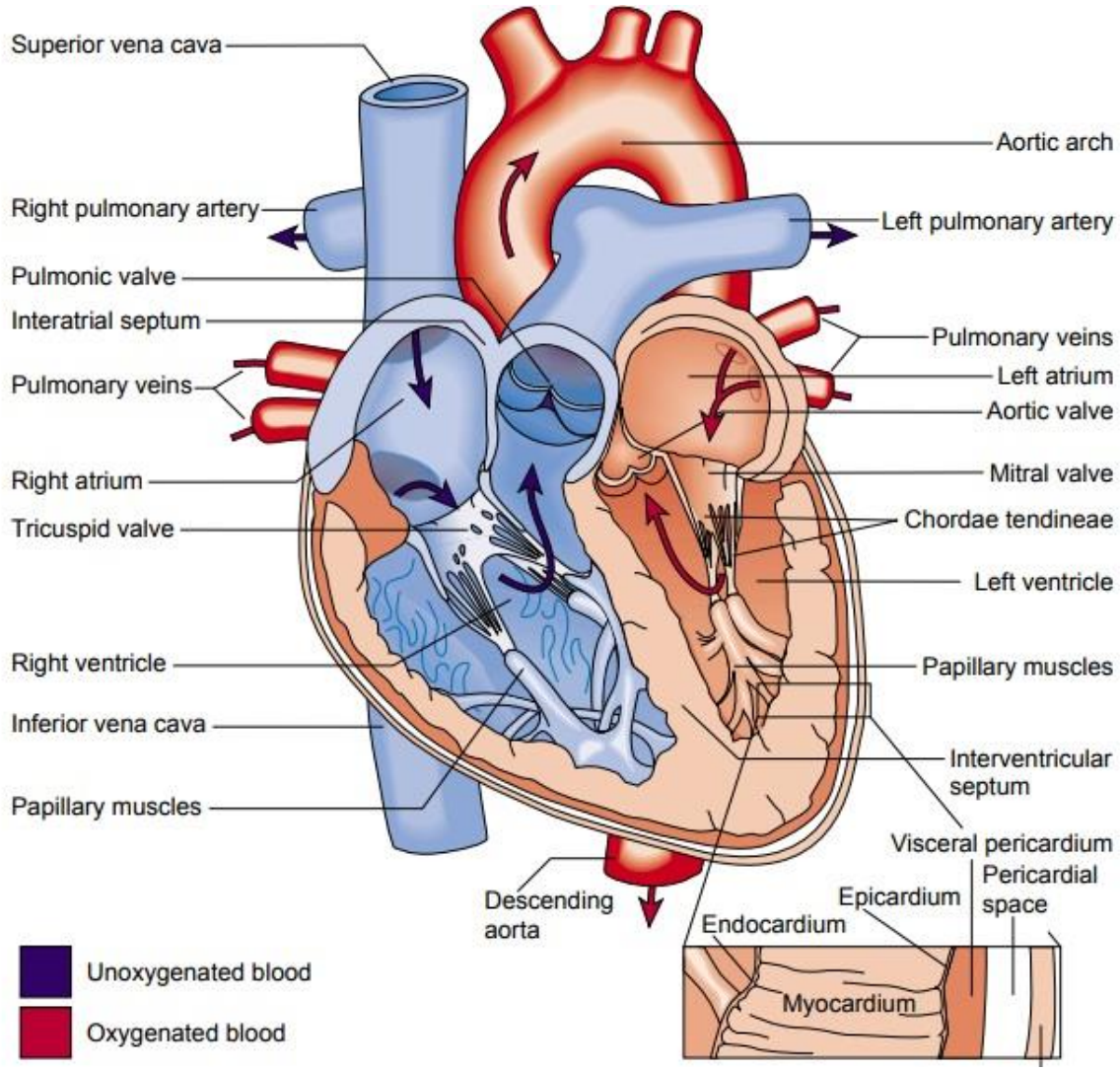


Figure 1.1: The basic anatomy of the heart is indicated, including chambers, major blood vessels, and conducting system (yellow), including the sinoatrial node, atrioventricular node, and Purkinje fibers (2)

The heart is encased in a thin, fibrous sac called the pericardium, which is composed of two layers. Adhering to the epicardium is the visceral pericardium. Enveloping the visceral pericardium is the parietal pericardium, a tough fibrous tissue that attaches to the great vessels, diaphragm, sternum, and vertebral column and sup-ports the heart in the mediastinum. The space between these two layers is filled with about 30 mL of fluid, which lubricates the surface of the heart and reduces friction during systole. The four chambers of the heart constitute the right-

and left-sided pumping systems. The right side of the heart is made up of the right atrium and right ventricle, distributes venous blood to the lungs via the pulmonary artery for oxygenation. The right atrium receives blood returning from the superior vena cava inferior vena cava and coronary sinus. The left side of the heart is composed of the left atrium and left ventricle, distributes oxygenated blood to the remainder of the body via the aorta. The left atrium receives oxygenated blood from the pulmonary circulation via the pulmonary veins (3).

The varying thicknesses of the atrial and ventricular walls relate to the workload required by each chamber. The atria are thin-walled because blood returning to these chambers generates low pressure. In contrast, the ventricular walls are thicker because they generate greater pressures during systole. The right ventricle contracts against low pulmonary vascular pressure and has thinner walls than the left ventricle. The left ventricle, with walls two-and-a-half times more muscular than those of the right ventricle, contracts against high systemic pressure (4)

Since the heart lies in a rotated position within the chest cavity, the right ventricle lies anteriorly and the left ventricle is situated posteriorly. The left ventricle is responsible for the apex beat or the point of maximum impulse (PMI), which is normally palpable in the left midclavicular line of the chest wall at the fifth intercostal space. The four valves in the heart permit blood to flow in only one direction. The valves, which are composed of thin leaflets of fibrous tissue, open and close in response to the movement of blood and pressure changes within the chambers. There are two types of valves: atrioventricular and semilunar. Although the SA node activity sets up the heart rate, the autonomic nervous system modulates this heart rate as well as the electrical conduction and contraction of the heart. Electrical activities of the heart can be precisely measured and monitored via

electrocardiography (ECG). Disturbances of the heart electricity result in various types of arrhythmias which can lead to serious consequences including death(5).

1.2.Cardiac arrhythmia

Arrhythmia is a heart rhythm disorder in which the heartbeat is irregular, too slow, or too fast. Arrhythmias that lead to slower heart rates are called bradycardias while those that lead to faster rates are called tachycardias. In general, arrhythmias result from abnormalities in impulse initiation, impulse conduction, or a combination of both (6).

Abnormal impulse initiation can be caused by either automaticity or triggered activity in the heart, while abnormal impulse conduction could lead to reentry. Reentry is a condition in which the excitation travels in a circular path and re-excites the same region more than once. Reentry could have a relatively fixed reentrant pathway and persist to excite the heart at a fast rate. This condition is dangerous because it may not permit the heart to pump enough blood and often degenerates into a chaotic state called fibrillation that involves pathways that continuously change their size and location. Ventricular fibrillation is lethal as the heart loses its ability to pump sufficient blood. Formation of a reentrant circuit requires presence of a unidirectional block as well as a pathway that produces enough conduction delay for the wave to re-excite the tissue following the refractory phase. The latter condition implies that the pathway be greater than the wavelength, in which the wavelength is the distance traveled by the wave during the functional refractory period. In normal heart the wavelength is often longer than any regular pathway in the heart. However, in diseased conditions, the wavelength could decrease due to electrophysiological remodeling that leads to conduction slowing, which could further lead to the formation of reentry (7). as show in (Figure 1.2)

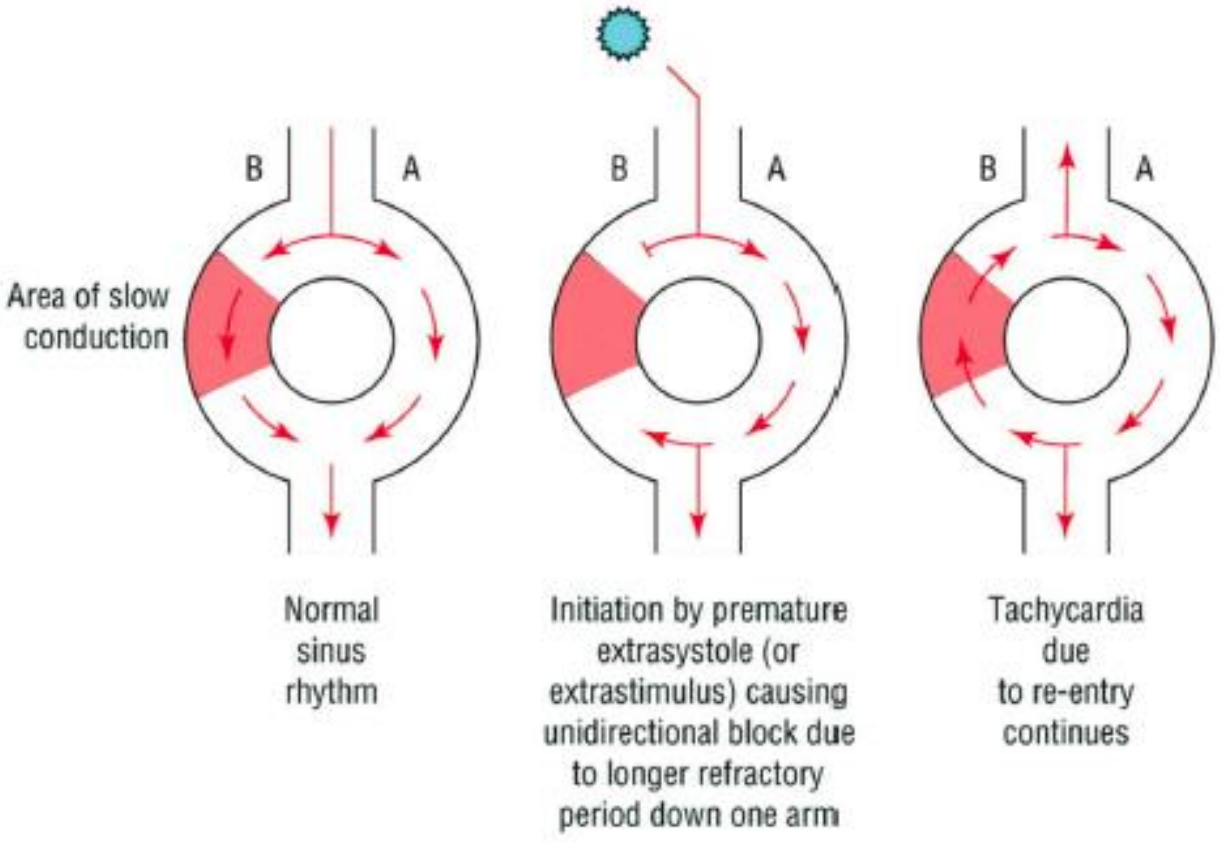


Figure 1.2: Mechanism of reentry circuit (7)

Cardiac arrhythmias are alterations of the normal cardiac rhythm, and the sequence of activation. During an arrhythmia episode, the heart rate can increase (tachycardia, above 100 beats/min), decrease (bradycardia, below 60 beats/min) or became irregular. Particular interest for this thesis is idiopathic ventricular tachycardias (VTs) which are tachycardias originated in ventricles with the absence of any significant structural disease. The most common way to diagnose an arrhythmia is by evaluating the patients ECG (8).

1.3.Types of Arrhythmias

Among all types of CVDs, arrhythmias are mostly responsible for sudden deaths and is indication of other high-risk diseases (9). Cardiac rhythm disorders cause an estimated 400,000 deaths annually in the United States alone, and about 7 million deaths worldwide (10). Clinical and experimental studies reveal basic electrophysiological differences between genders, which likely reflect the occurrence of arrhythmias (11). Arrhythmia is the collective term for a variety of conditions that involve a heart rhythm other than sinus rhythm (12).

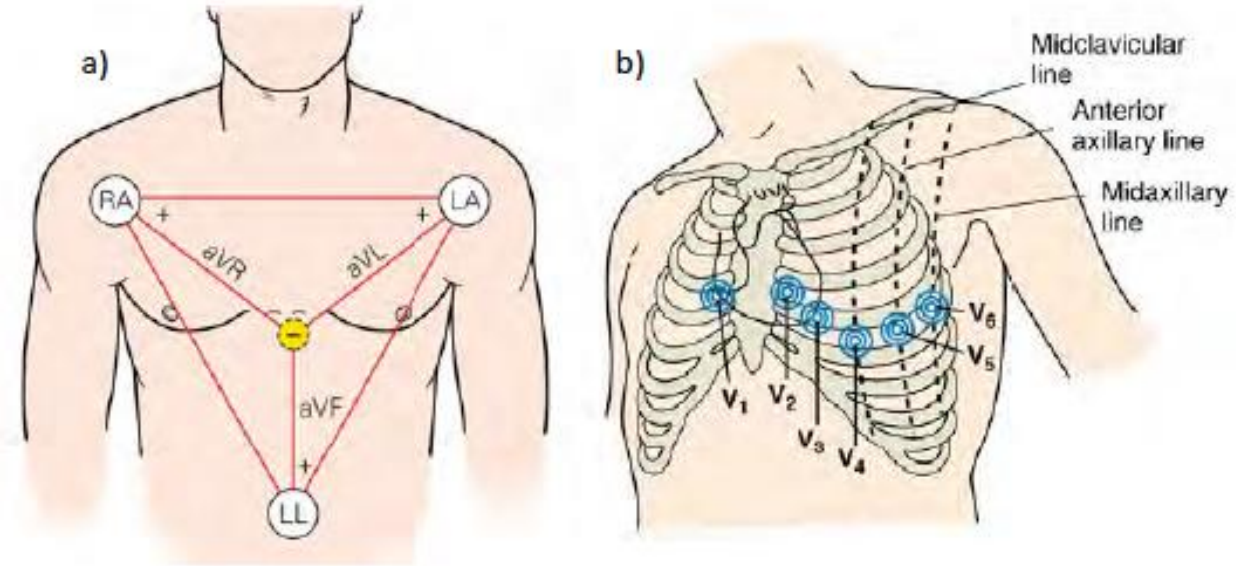


Figure 1.3: a) Bipolar limb leads and augmented unipolar leads. Electrode location is also shown. b) Precordial leads electrode placement. Reproduced, adapted a modified from (11).

Arrhythmia symptoms can range from normal and harmless symptoms to life-threatening symptoms of serious heart disease, such as cardiac arrest. Arrhythmias may be classified to several ways based on different factors. They can be categorized based on heart rate, bradycardia or tachycardia. Some arrhythmias are classified based on their cause, which can include medical conditions such as

hyperthyroidism, severe anemia, or lack of oxygen. Arrhythmias can be categorized as regular or irregular. An arrhythmia can also be classified based on the foci in the heart from which it originates for example, an arrhythmia originating at the ventricular level is called a ventricular arrhythmia, while one located in the atrium is termed a supraventricular arrhythmia. Arrhythmias that occur due to changes in the heart, are called organically caused arrhythmias, while those due to changes in the autonomic system are termed functionally related arrhythmias. Finally, some arrhythmias are caused by certain pharmaceuticals, such as digitalis, diuretics, certain antiarrhythmic drugs, and some psychotropic drugs (13).

1.3.1. Atrial fibrillation

Atrial Fibrillation (AF) is the most common sustained cardiac arrhythmia and is associated with significant morbidity. It is characterized by uncoordinated atrial activation and subsequent deterioration of atrial mechanical function(14).

AF involves complete absence of SA note stimulus. Under normal conditions, SA node stimulus depolarizes the cells and causes a wave of contraction throughout the atria. Upon opening of the valves between the atria and 15 ventricles, up to 70% of blood within the atria falls into the ventricles, aided by gravity.

The final 30% of blood is pushed from the atria into the ventricles by atrial contraction, known as the “atrial kick”. In AF, the atria is fibrillating, i.e. quivering, and the atrial kick does not occur, resulting in an at least 30% loss of blood to the arterial and coronary circulations (13).

On ECG , AF is characterized by the replacement of regular P-waves with rapid oscillations or fibrillatory waves that vary in amplitude, shape, and timing, and is

associated with irregular and often rapid ventricular responses when AV conduction is intact. The European Society of Cardiology (ESC) guidelines (14), providers the following consensus statement regarding simple and clinically useful definitions of AF:

- Paroxysmal AF: If the arrhythmia converts spontaneously within 7 days (most often within 24 hours).
- Persistent AF: If the arrhythmia lasts longer than 7 days but is converted by either pharmacological or direct-current cardioversion.
- Permanent AF: Long-lasting arrhythmia that does not respond to cardioversion or for which cardioversion has not been attempted.
- Recurrent AF: When a patient has had more than two AF episodes. Both paroxysmal and persistent AF can be recurrent.
- Lone AF: No universal definition exists, but usually refers to AF in the absence of any clinical or echocardiographic evidence of cardiopulmonary disease, including hypertension and diabetes, and any other known precipitating cause or illness.
- Non-valvular AF: AF in the absence of rheumatic mitral valve disease, prosthetic heart valve, or mitral valve repair.

1.3.2. Supraventricular tachycardia (SVT)

It is a heterogeneous group of arrhythmias used to describe tachycardias that involve cardiac tissue at the level of the bundle of His or above. The prevalence of SVT is 2.25% in 1000 persons with a female predominance of 2:1 across all age groups(15). SVT increases patient morbidity, particularly when symptoms are frequent or incessant, and in a small cohort of patients with atrial fibrillation (AF)

and ventricular pre-excitation, it can be life-threatening. It is a common cause of hospital admissions and can cause significant patient discomfort and distress. The most common SVTs include atrioventricular nodal re-entrant tachycardia, atrioventricular re-entrant tachycardia and atrial tachycardia. In many cases, the underlying mechanism can be deduced from electrocardiography during tachycardia, comparing it with sinus rhythm, and assessing the onset and offset of tachycardia. Supraventricular arrhythmias have a narrow QRS complex because there's a rapid excitation of the ventricles, which means the arrhythmia is originating above or within the bundle of His, as shown in figure (1.4) (16).

In the normal electrical conduction pathway of the heart, An ECG tracing specifically shows how the depolarization wave flows through the heart during each heartbeat. The normal electrical activity of the heart starts in the sinoatrial or SA node and is then conducted through the atrium, creating the P wave on ECG.

From the atrium, electrical activity goes to the atrioventricular, or AV node, after which it goes through then the right and left branches of the Bundle, and finally through the Purkinje fibers, which deliver the current to the right and left ventricles.

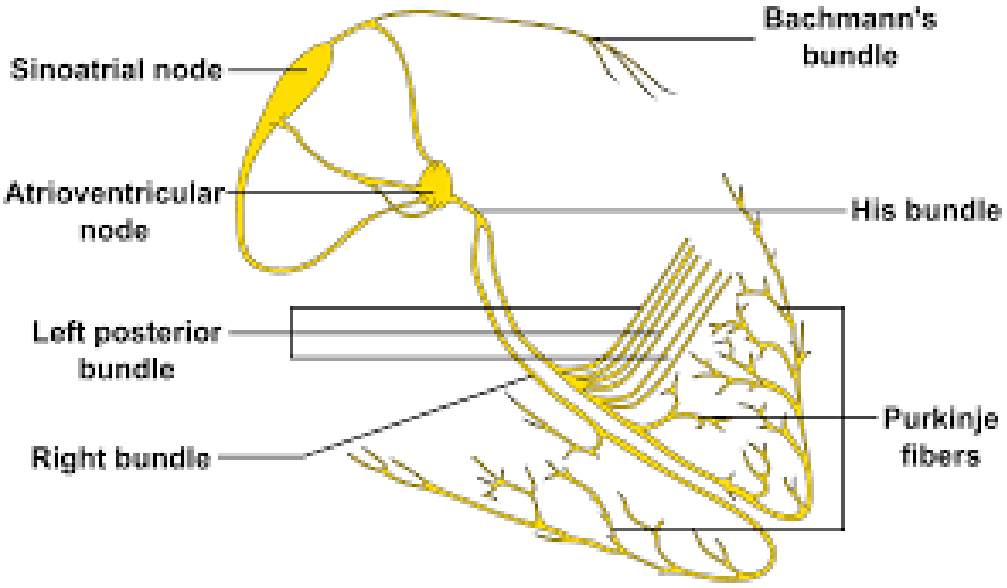


Figure 1.4: Heart conduction system (16)

1.4.Risk factors of arrhythmia:

Early life factors may be involved in the pathogenesis of arrhythmia (17). Obesity was associated with an increased arrhythmia risk in community- and population-based cohort studies, and in cardiothoracic surgery cohorts, an independent type of cardiac surgery(18).

Adipose tissue itself may be directly involved in the pathogenesis of cardiovascular disease, considering that obesity has been associated with generalized enlargement of fat depots, which is involved in the production of pro-inflammatory cytokines and reactive oxygen species and uncontrolled release of fatty acids (18). Cardiac adiposity is characterized by an increase in intramyocardial triglyceride content and an enlargement of the fat tissue surrounding the heart and vessels, which can lead to myocardial damage. Fatty acid infiltration and overload promotes fatty acid oxidation, accumulation of triglycerides and metabolites which can impair calcium signaling, beta-oxydation and glucose utilization, damage mitochondrial function

with increased production of reactive species, proapoptotic and inflammatory molecules (20). Fatty infiltration or "fatty metamorphosis" can induce abnormal automaticity from degenerated myocardial cells (21).

Obesity is an established inflammatory condition (22), while adipocytes enable local inflammation through adipocytokines and proinflammatory cytokines (23). Inflammation, measured by plasma levels of high sensitivity C reactive protein, fibrinogen and soluble intracellular adhesion molecule-1, was significantly associated with the risk of incident atrial fibrillation (24). Chronic inflammation may induce electrophysiological and structural changes in the atrial myocardium predisposing patients with triggering atrial foci to atrial fibrillation(25). Proposed mechanisms linking inflammation and arrhythmia include endothelial dysfunction, production of tissue factor from monocytes, increased platelet activation, and increased expression of fibrinogen (26). It is still not clear if inflammatory markers elevation is a consequence or a cause of arrhythmia. Probably preexisting inflammation initiates the arrhythmia that subsequently propagates an inflammatory response, enabling persistence of atrial fibrillation (27).

Adiponectin is one of the adipocytokines secreted by the adipose tissue, both a biomarker and a possibly mediator of cardiovascular disease, with antiatherogenic, antidiabetic and anti-inflammatory properties are able to influence the extent of atrial and left ventricular remodeling, which can increase cardiac contractility and action potential duration by inhibiting delayed rectifier potassium currents. Higher adiponectin levels were detected in patients with chronic atrial fibrillation than in paroxysmal atrial fibrillation, correlated with a collagen type I degradation marker, demonstrating that adiponectin is a useful marker of atrial remodeling. Activation of fibroblasts and subsequent fibrosis contribute to atrial structural remodeling and heterogeneity of the cardiac conduction tissue(28).

Epicardial fat tissue modulates atrial electrophysiological and contractive properties, through inflammatory cytokines, adipocytokines and adipocyte-cardiomyocyte interactions, and heart failure epicardial fat that has a greater arrhythmogenic effect on the left atrium, prolonging action potential duration (29). Epicardial adipose tissue was also suggested to be involved in the maintenance of atrial fibrillation (30). The right atrium is more resistant to hypoxia/ reoxygenation than the left atrium, due to higher heat shock proteins (31). Several experiments showed that epicardial adipocytes modulate atrial cardiac ionic currents with decrease of delayed rectifier inward and outward currents and increase of late sodium currents and L-type calcium currents (32).

Increased intracellular lipid content can impair repolarization due to a decrease in potassium channel protein levels, causing ventricular tachycardia and sudden cardiac death (33). Adipocytokines from epicardial fat significantly decrease delayed rectifier outward currents in cardiomyocytes, prolonging action potential duration and facilitating triggered activity with early after depolarizations (34).

Weight gain delays cardiac repolarization and several studies reported prolonged QT intervals in obese patients, including persons with uncomplicated obesity and abdominal fat deposition (35). Other studies that were conducted in patients with uncomplicated obesity, reported no effect of weight gain on cardiac repolarization (36).

Obese subjects exhibit increased systemic oxidative stress, enhanced by abdominal adiposity and associated with adiponectin deficiency(37). Recent clinical and experimental evidence demonstrated the involvement of oxidative stress in cardiac electrical and structural remodeling (38). Reactive oxygen species may impair Na, K and Ca channels, Na-Ca exchanger activity while may be implicated in gap

junction remodeling, decrease the action potential amplitude and duration, and increase the incidence of cardiac arrhythmias in animal models (39).

Oxidative stress results in decreased hERG protein levels, accelerated activation and deactivation of hERG, and increase in current amplitude of hERG and hKv1.5, allowing a greater amount of K ions to flow through these channels in the phase 3 of the action potential, downregulation of Ito (responsible for the rapid repolarization phase), and increases the channel opening probability of Ik1 (inward rectifying channel) (38). An abundance of oxidative markers was found in atrial tissues from patients with persistent atrial fibrillation, although plasma markers of oxidative stress did not correlate with developing atrial fibrillation (40).

1.5.Arrhythmia detection Tools

1.5.1. Non-Biochemical tools

1.5.1.1. Electrocardiography (ECG)

In general, the approaches to arrhythmia beat detection, degree assessment, and therapy selection are based on ECG—a noninvasive and low-cost method of obtaining clinical information regarding the heart by measuring the skin surface potential. Morphological changes on the ECG waveform can reveal abnormal cardiac beats, helping in arrhythmia detection (41).

1.5.1.2. Long-term recording ECG (LTRE)

For decades, the 24-h Holter monitor has been the gold standard method for investigating patients with suspected arrhythmias, including AF, in the ambulatory setting. Many studies demonstrate the benefits of this method; however, there are some limitations regarding its use and applicability. When using a Holter monitor, the diagnostic yield ranges from only 15–39% because intermittent periods of paroxysmal AF that do not manifest during the 24-h monitoring period remain unnoticed (42). The highest diagnostic yield is achieved with implanted devices, such as loop recorders (43). However, such monitors are costly, invasive, and limited to a certain subset of patients. The monitored data are collected by the sensor and wirelessly transferred (44).

1.5.1.3. Magnetic Resonance Imaging

Magnetic Resonance Imaging (MRI) is a non-invasive imaging technique widely used in biomedical research and clinical settings. MRI is capable of providing exceptional soft tissue contrast in the body's organs such as the brain, liver and heart. The basic principle of signal detection in MRI relies on the absorption and emission of radiofrequency (RF) energy by the atomic nuclei in the presence of an external magnetic field. The majority of the researches and clinical MRI techniques use the hydrogen atoms naturally present in the water molecules inside the body to generate a radiofrequency signal. Typical MRI hardware consists of an MRI magnet to provide a strong static magnetic field, while radiofrequency coils are responsible for transmitting and receiving the signal from the object, and gradient coils to create a spatially variant magnetic field. The generation of spatial images in MRI involves manipulation of nuclear magnetic spins present in the object using an external field, while the detailed interaction of the spins with other spins and the external field lies in the realm of quantum physics. Macroscopic phenomenological models of spin magnetization are usually sufficient to describe

such interactions in the context of MR pulse sequence design. At the equilibrium state, the magnetic spins are aligned with the direction of the external static magnetic field (45).

1.5.2. Biochemical tools

Adipose tissue is a highly active organ that is now recognized to be an active participant in energy homoeostasis and physiological functions such as immunity and inflammation. Adipose tissue is known to express and secrete a variety of products known as “adipokines”, including leptin, adiponectin, resistin and visfatin. The release of adipokines by either adipocytes or adipose tissue-infiltrated macrophages leads to a chronic sub-inflammatory state that could play a central role in the development of many associated cardiovascular disease (46).

The adipocyte and its secretory products have been implicated in a wide variety of physiological processes. Adipose tissue produces and secretes hormone-like signaling molecules, cytokines and adipokines impacting multiple target organs (47). Moreover, it expresses a wide range of receptors, which causes to respond to numerous metabolic and endocrine stimuli involved in modulating blood pressure, glucose metabolism, inflammation and atherosclerosis (48).

1.5.2.1. Chemerin

Chemerin, a chemokine highly expressed in liver and white adipose tissue, was initially described as a protein with a complex immune system function. Parallel lines of investigation also support the notion that chemerin is a novel adipokine that regulates adipocyte development and metabolic function as well as glucose metabolism in liver and skeletal muscle tissues. A growing body of human experimental data indicates that serum chemerin levels are elevated in

patients with obesity and that they exhibit a positive correlation with various aspects of the metabolic syndrome. Thus, the role of chemerin in inflammation and metabolism might provide a link between chronic inflammation and obesity, as well as obesity-related disorders such as type 2 diabetes and cardiovascular disease. chemerin expression and secretion have been shown to increase dramatically with adipocyte differentiation (49)(50).

1.5.2.1.1. Structure and processing

Chemerin is translated as a 163 amino acid pre-proprotein that is secreted as a 143 amino acid (18 kDa) proprotein following proteolytic cleavage of a signal peptide (51). (figure 1.5). The figure shows the proteolytic processing of pre-Prochemerin (1–163) into mature Prochemerin (21–163) after the removal of N-terminal signal peptide. Cathepsin G removes seven amino acids from C- terminal end, resulting in the formation of Chemerin (21–156); elastase forming Chemerin (21–157) after the removal of six amino acids, Chemerin (21–155) after removing eight amino acids, and Chemerin (21–152) after removal of eleven amino acids. Plasmin removes five amino acids and forms Chemerin (21–158), and tryptase cleaves five and eight amino acids resulting in formation of Chemerin (21–158) and Chemerin (21–155) respectively. Prochemerin undergoes multiple cleavages, such as tryptase cleavage forming less active Chemerin (21–158) which then undergoes further cleavage by CPN or CPB resulting in Chemerin (21–157) formation. Active Chemerin (21–156) and Chemerin (21–157) peptides undergo further cleavage(s) resulting in the formation of inactive Chemerin fragments.

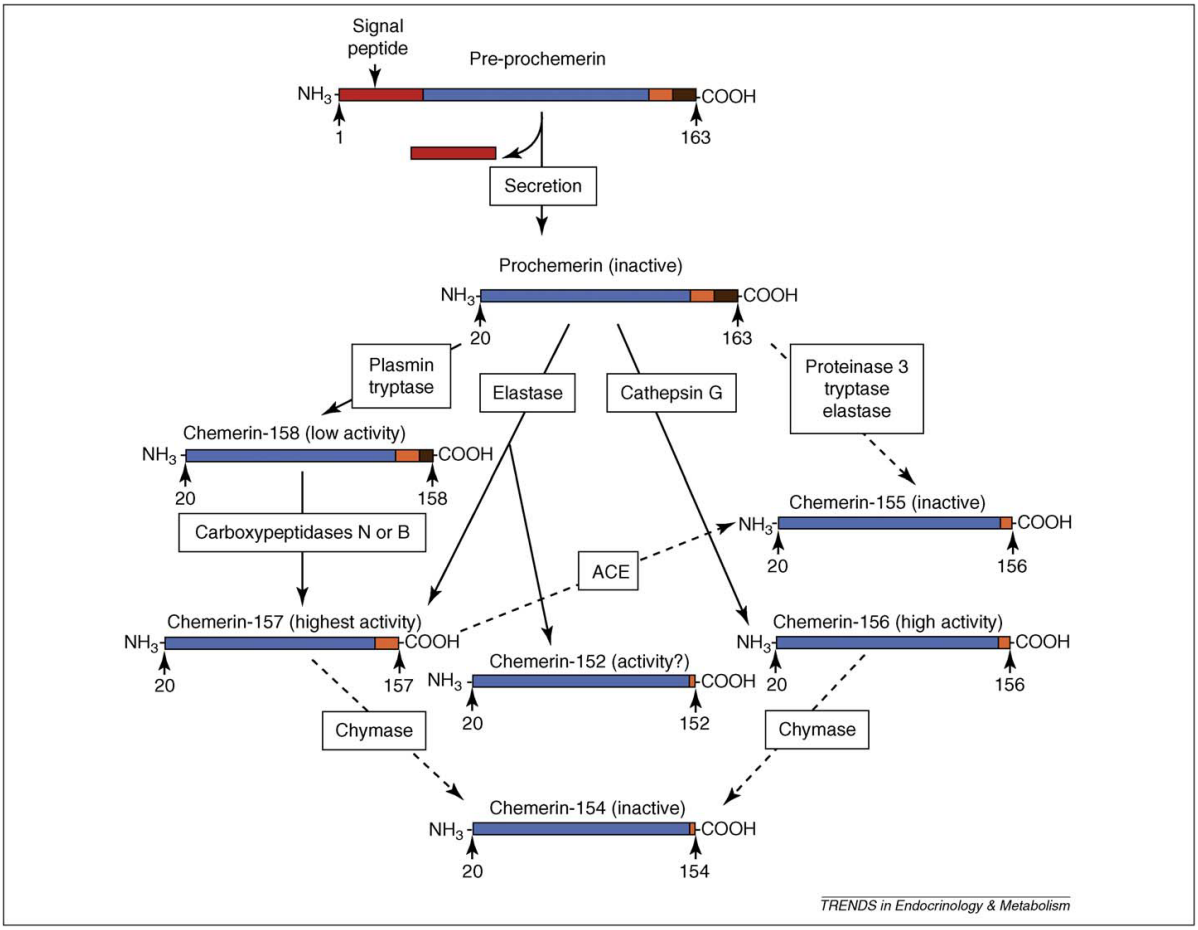


Figure 1.5: Chemerin proteolytic processing scheme (51)

This proprotein has low biological activity, and requires further extracellular C-terminal processing by plasmin, carboxypeptidases or serine proteases of the coagulation, fibrinolytic and inflammatory cascades (52).

Interestingly, the extent of C-terminal cleavage depends on the location from which chemerin is isolated. For example, chemerin from human ovarian ascites fluid, serum and hemofiltrate lacks six, eight and nine C-terminal amino acids, respectively (53). These findings are consistent with observations that several enzymes are capable of processing chemerin to an active form. Vitro studies have shown that cathepsin G cleaves seven C-terminal amino acids from prochemerin, elastase is able to cleave six, eight or eleven, plasmin cleaves five, and tryptase cleaves five or eight (54).

In some cases, multiple cleavages are required to fully activate chemerin. For example, an initial tryptase cleavage at amino acid 158 results in chemerin with very low activity.

However, this product serves as a substrate for a second cleavage by carboxypeptidase N or B, producing fully activated bioactive chemerin (55). Proteolytic processing is also believed to be involved in the inactivation of chemerin. In particular, neutrophil-derived serine protease proteinase, mast cell chymase and angiotensin-converting enzyme (ACE) have been shown to convert bioactive forms of chemerin to inactive derivatives (56). Thus, proteolytic processing of chemerin is a key regulatory mechanism that might determine both systemic and local concentrations of bioactive chemerin. A key area of research is to elucidate the physiologic and pathophysiologic relevance of chemerin processing. Interestingly, many of the known biological activities of chemerin can be largely recapitulated by small peptides that are identical to or are synthetic variations of the 9–15 C terminal amino acids of chemerin 20–157 (57). Although an unlikely proposition, this suggests that the remaining portion of the protein is dispensable and devoid of function.

1.5.2.1.2. Chemerin in cardiovascular disease

Chemerin performs various functions as a growth factor, chemokine and as an adipokine in vascular disease complications. Ferland and Watts proposed that Chemerin may be considered a growth factor in addition to its previously known chemo- and adipokine-like functions (58).

As a growth factor, Chemerin induces Matrix Metalloproteinase (MMP)-2 and -9 activities causing growth and remodelling of blood vessels, including EC proliferation, migration, and angiogenesis. Angiogenesis is another well-known and essential process in EC biology which is tightly regulated in fetal vasculogenesis and EC formation. A research group reported for the first time that Chemerin increased angiogenesis in microvascular ECs in a time- as well as in a dose-dependent manner, further implicating its role in human vasculature. Acting via MMP2 and -9 proteinases, Chemerin further plays an important role in vascular remodelling and regeneration (59). CMKLR1 is selectively present on the cells of the immune system and Chemerin-CMKLR1 binding stimulates the receptor and promotes chemotaxis of all leukocyte cell populations expressing CMKLR1 receptor (60).

Additionally, Hart and Greaves reported that Chemerin promotes macrophage adhesion to extracellular matrix protein fibronectin and Vascular Cell Adhesion Molecule (VCAM)-1. It also encourages the clustering of Very Late Antigen (VLA)- 4 and -5 integrins (61). Chemerin stimulates an increase in intracellular Ca2+ concentrations, activates Nuclear Factor-kappa (κ) B and Matrix- activated Protein Kinases (MAPK) pathways in monocytes, macrophages (62).

To date, it remains inconclusive if Chemerin is a pro-, inflammatory, and/ or an anti-inflammatory molecule as literature appear to show that it depends upon the ‘type and number of enzyme(s)’ involved in Chemerin cleavage(s), and/or Chemerin peptide(s) binding to one or more receptors (63).

Overall, Chemerin forms a significant connecting link among obesity, inflammation and atherosclerosis in humans. Chemerin is known to play a significant role in the amalgamation of dysfunctional metabolic states which

further contribute to CVD, with obese patients particularly carrying ten times higher circulating Chemerin levels compared to healthy counterparts (64), as shown in figure (1.6)

Circulating Chemerin levels positively correlate with various factors of MetS such as high circulating glucose levels, triglycerides, high blood pressure and low levels of High-density Lipoprotein (HDL), and high Low-density Lipoprotein (LDL) levels (65).

Additionally, Manco and colleagues reported that Chemerin, similar to traditional markers of arterial stiffness and carotid intima-media thickness (CIMT) including non-HDL-cholesterol and Triglyceride/HDL ratio which was associated with these risk factors (66). Furthermore, Salama and colleagues reported that these two markers also positively correlated with Chronic Kidney Disease (CKD), signifying subclinical atherosclerosis, with Chemerin emerging as an independent predictive marker for atherosclerosis (67).

Increased serum Chemerin levels are reported to be positively correlated with presence of atrial fibrillation, with patients diagnosed with permanent atrial fibrillation having highest serum Chemerin concentrations compared to patients with persistent and paroxysmal atrial fibrillation (68). Inci S and colleagues reported that Chemerin to be an independent predictor of CV events (69).

Plasma Chemerin levels independently predict a significantly increased risk of future CV events in coronary patients. Hence, the proposition of Chemerin as a new risk factor in the development of CVD. In addition to the above cardiovascular functions, multiple, recent studies propose using circulating

Chemerin concentration as a novel predictive biomarker in dilated cardiomyopathy, contributing significant evidence toward Chemerin role in CVD (70).

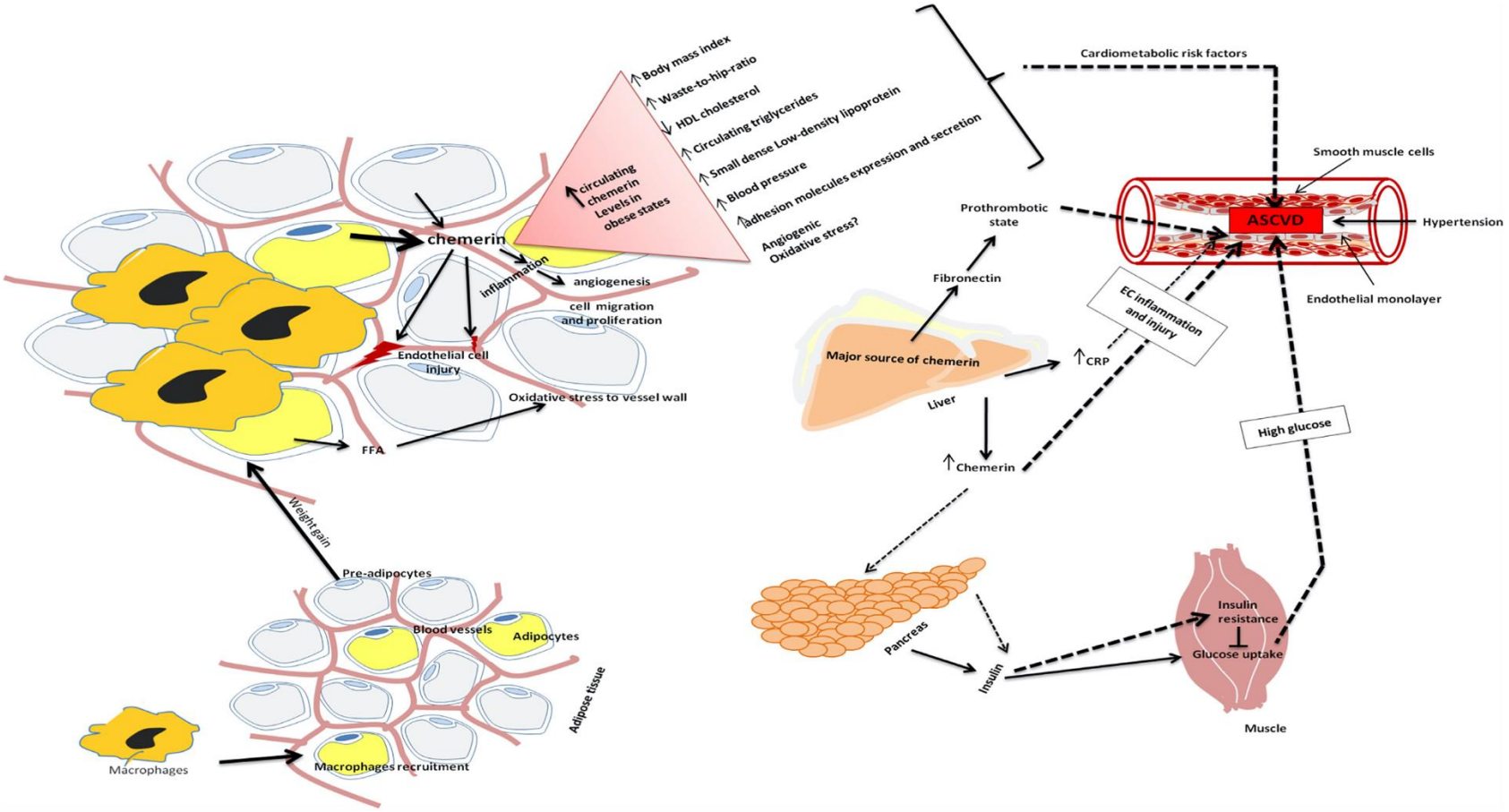


Figure (1.6) Abnormal adipose tissue inflammation along with macrophage infiltration and increased Chemerin production in obese adipose tissue. The figure also illustrates the relationship(s) between altered secretion of various other metabolic risk factors and abnormal Chemerin secretion by the adipose tissue. These various metabolic risk factors further elicit their effects in peripheral organs including vascular cell wall. (64)

1.5.2.2. Nesfatin-1

Nesfatin-1 is an 82-amino acid peptide highly expressed in several regions of the hypothalamus and later in peripheral tissues. It is derived from the protein nucleobindin-2 (NUCB2) (71). It exerts a large array of behavioral effects and acts as an integral regulator of energy balance, circadian feeding rhythm, and related endocrine functions(72). In the cardiovascular system, central Nesfatin-1 activates the nervous circuits that are responsible for hypertension (73). Endogenously expressed Nesfatin-1 was recently identified in the heart of mammals (74), suggesting that Nesfatin-1 may be an endogenous modulator of cardiac performance (75). However, the mechanisms underlying these effects of Nesfatin-1 in cardiomyocytes remain unknown.

It is newly discovered as a adipokine, inhibits inflammatory response which is involved in the mechanism of atrial fibrillation (AF). Inflammation is closely correlated with AF development. AF prevalence was significantly higher in individuals with inflammatory conditions (76).

Nesfatin-1 was originally identified as a neuropeptide of the hypothalamus, which is a key integration area of the brain, where numerous neuropeptides and transmitters released to participate in the control of essential body functions (77). Nesfatin-1 signaling in the brain has also been involved in the modulation of cardiovascular responses potentially involved in stress adaptation, such as causing an increase in mean arterial pressure in rats (78).

Electrophysiological analyses demonstrated that nesfatin-1 hyperpolarizes the neuropeptide-Y (NPY) neurons in the arcuate nucleus (79), suggesting that the anorexigenic action of the peptide was due to an inhibition of the activity of the

neurons producing the orexigenic, NPY. Nesfatin-1 directly affects the membrane potential of the paraventricular neurons. It has been shown recently that nesfatin-1 immunoreactivity can be detected throughout the hypothalamus in neurons that produce vasopressin, oxytocin, melanin-concentrating hormone, and somatostatin (80).

Neuropeptide-Y is released at the sympathetic nerve terminals of the heart and inhibits vagal stimulation. It was shown that nesfatin-1 inhibits the NPY neurons in the arcuate nucleus (81).

The nesfatin-1 is involved in several inherited diseases collectively known as cardiac channelopathies. These cardiac disorders are manifested with different electrocardiographic patterns and sometimes with different clinical features, although they are often characterized by similar arrhythmic events. L-type voltage-gated Ca^{2+} channels (VGCC) are voltage-dependent channels that open in response to membrane depolarization, permitting entry of Ca^{2+} into the cell (82).

The depolarizing current through L-type VGCC contributes to the plateau phase of the cardiac action potential as well as to pacemaker activity in nodal cells (83). The influx of Ca^{2+} subsequently triggers the release of intracellular Ca^{2+} stores from the sarcoplasmic reticulum, and the ensuing intracellular Ca^{2+} transient results in the activation of the myofilaments, allowing cell contraction (84).

Additionally, L-type VGCC can affect other cellular processes modulated by intracellular Ca^{2+}, including gene expression and excitation-secretion coupling (85). Therefore, alterations in density or function of L-type VGCC have been implicated in a variety of cardiovascular diseases, including atrial fibrillation, heart failure, and ischemic heart disease (86).

Altering the properties of the VGCC could have detrimental effects on cardiac electrical and contractile functions (87). Importantly, these channels are modulated by a variety of hormones, neurotransmitters, and cytokines, operating via G-protein coupled receptors and second messengers, and thereby profoundly affecting the functions of target tissue (88).

1.6. Knowledge gap

A cardiac arrhythmia simply is defined a variation from the normal heart rate and/or rhythm that is not physiologically justified. The recent years have witnessed important advances in our understanding of the electrophysiologic mechanisms underlying the development of a variety of cardiac arrhythmias. Adipokines are produced from adipose tissues, which are considered endocrine organs involved in cardiovascular function causing electrophysiological effects such as ionic profiles, contractility in the atrium, and change in morphology of action potential. Also, adipokines are involved in cardioprotective effects (89).

In addition, adipokines are CVD mediators or biomarkers that affect the heart as well as blood vessels, by increasing the cardiac contractility and action potential duration, which result in the extent of left ventricular and atrial remodeling (90).

Circulating biomarkers related to inflammation, neurohormones, myocardial stress, and necrosis have been associated with commonly encountered arrhythmic disorders. Both direct and indirect biomarkers implicated in the heart diseases have potential prognostic value in such patients. The knowledge gap regrarding the linke between the propsed bioomarker and their role in the cardiac conduction in arrythmia cases would be covered in this study.

The current knowledge of causes and idiopathic Arrhythmia is limited. We aimed to investigate the nature of abnormalities including clinical assessment and early

diagnosis by studying the association between serum nesfatin-1 & Chemerin concentrations in patients with SVT arrhythmia

1.7.Implications and contribution to knowledge

It has been reported that Certain adipokines are strongly involved in the heart contraction. Serum nesfatin-1 & chemerin are a novel prognostic indicator of major adverse cardiac events. Circulating chemerin can improve early risk stratification for Arrhythmia patients. This study might highlight the relevance of these adipokines within cellular environment and link with cardiac channelopathies disease (91).

Increasing literary evidence suggests that Chemerin is strongly associated with markers of inflammation. To date, the ambiguous role of Chemerin in inducing 'inflammation' is still debatable with divided opinions.
In addition, along with studying additional Chemerin levels in human health and disease, more research is required to understand their role. No previous study was linked nesfatin-1 & chemerin with SVT arrhythmias.

1.8.Aims of the study

The current knowledge of causes and idiopathic Arrhythmia is limited. This study would aim to investigate the nature of abnormalities including clinical assessment and early diagnosis by:

- Investigating the diagnostic value of serum nesfatin-1 & Chemerin levels in patients with SVT arrhythmia
- Studying the correlation of proposed adipokines with lipid profile in patient

- Performance of ROC analysis and identify the cutoff values of these biochemical markers in early diagnosis of SVT cases.

Chapter Two

Materials & Methods

2. Materials and methods

2.1.Study design

The present work included a case control study for a group of (90) samples: (60) patient samples , (30) healthy control samples. The study was conducted from October 2021 to October 2022.

Patients with (SVT with risk factor and SVT without risk factor) etiology Arrhythmia cases (SVT)
were selected from the cardiac disease centre / Al Hussein Teaching Medical Hospital. The questionnaire used in this study (appendix I) was developed based on literature review and discussion between the researcher and supervisors team.

To reduce potential bias introduced by self-reported data, participants were ensured on the confidentiality and privacy of their responses. They were also exposed to medical examination for signs and symptoms of any cardiac disorders manifest with electrocardiographic patterns by specialized doctor.

The questionnaire was structured into different sections. The sociodemographic characteristics of the participants were obtained and represented by the Baseline characteristics. Additionally, participants were asked to report their history with chronic conditions.

Also, the questionnaire was involved in a section for collecting data about Comorbidities at sudden cardiac arrest, Baseline electrocardiographic parameters, Medication, etiology and Blood biochemical tests.

2.2.Materials

2.3.Instruments:

In this chapter, instruments and tools were described and listed in table (2.1)

Table 2.1 The instruments used in the study

Instrument	Suppliers
Centrifuge	HETTICH/ Germany
Deep Freeze	COOLTECH/ China
ELISA system	UNO/HUMAN/ Germany
Roller Mixer	China

2.4. Tools, materials and Kits

The tools, materials and kits with their supplier which were used in this study are listed in table (2.2).

Table 2.2: Tools and materials used in the study

Tools and Materials	Suppliers
Pipette(100-1000µl)	DRAGON MED/ USA
Micropipette(10-100 µl)	DRAGON LAB/ USA
Gilson Tips,1000µl (blue)	China
Gilson Micro-tips, 100µl	China
Eppendroff Tubes	China
Gel tubes	China
Sodium citrate tubes	China
EDTA tubes	China
Gloves	China
Syringe	China

Table 2.3 kits which are used in this study

Kits	Suppliers
Chemerin Kit	Bioteck/ China
Nesfatin-1 Kit	Bioteck/ China
Lipid profile Kits	Bioteck/ China

2.5. Inclusion and Exclusion criteria:

2.5.1. Patients Criteria

All patients were subjected to the full clinical history, clinical examination, and relevant laboratory investigations. The diagnosis of the SVT clinical conditions was established according to the latest clinical practice guidelines by the WHO. The etiology of cases was identified based on evaluation of ECG and laboratory measurements for the clinical assessment.

2.5.2. Patients Exclusion criteria:

Patients with other types of Arrhythmia were excluded in this study.

2.5.3. Control Criteria:

Control group of an apparently healthy 60 subjects were chosen from well-known volunteers participants. Blood samples were drawn from the volunteers, participants who had no history of heart diseases. The ages of the participants were also convergent in the whole study group. Demographic information of the participants were also collected through the self- reported technique (student questionnaire) (appendix II).

2.6. Study variables:

- **Dependent Variable**

Serum nesfatin-1, Chemerin

- **Independent Variable**

Age, gender, smoking state.

2.7. Approval of the Ethical Committee

The protocol of the study was approved by Ethical Committee of Kerbala Medical College, and committee of heart disease unit in Al Hussein Teaching Medical City. Samples from serum were obtained after consent from patients or the patients' relatives.

2.8. Blood Collection and Storage

Blood samples were collected from heart disease center of Al Hussein Teaching Hospital. Five mL of blood samples were drown by venipuncture using 5mL disposable syringes, blood was left for (15 min) at room temperature in gel tube. Serums were separated by centrifuging for 10 minutes at approximately 4000 xg. Serum samples were aliquot into two Eppendorf and store at -20°C to avoiding multiple freezing-thawing cycles and used for the further measurement. Blood collection tubes were disposable, non-pyrogenic, and non-endotoxin.

2.9. Methods:

2.9.1. Measurement of chemerin levels in Human serum:

Principle: This ELISA kit uses Sandwich-ELISA as the method. The Microelisa stripplate provided in this kit has been pre-coated with an antibody specific to chemerin. Standards or samples are added to the appropriate Microelisa stripplate wells and combined to the specific antibody. Then a Horseradish Peroxidase (HRP)- conjugated antibody specific for chemerin is added to each Microelisa stripplate well and incubated.

Free components are washed away. The TMB substrate solution is added to each well. Only those wells that contain chemerin and HRP conjugated chemerin antibody will appear blue in color and then turn yellow after the addition of the

stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm.

The OD value is proportional to the concentration of chemerin. one can calculate the concentration of chemerin in the samples by comparing the OD of the samples to the standard curve.

Reagents :

The Measurement of chemerin levels method reagents were shown in table (2.4)

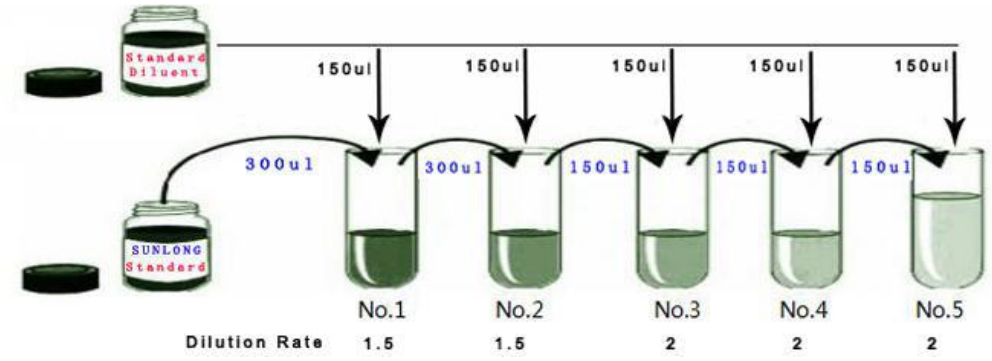
Materials provided with the kit	96 determinations	Storage
User manual	1	R.T.
Closure plate membrane	2	R.T.
Sealed bags	1	R.T.
Microelisa stripplate	1	2-8℃
Standard：2700 pg/ml	0.5mL×1 bottle	2-8℃
Standard diluent	1.5mL×1 bottle	2-8℃
HRP-Conjugate reagent	6mL×1 bottle	2-8℃
Sample diluent	6mL×1 bottle	2-8℃
Chromogen Solution A	6mL×1 bottle	2-8℃
Chromogen Solution B	6mL×1 bottle	2-8℃
Stop Solution	6mL×1 bottle	2-8℃
wash solution	20mL (30X)×1bottle	2-8℃

- **Preparing of Standard Curve:**

Dilute the standard by small tubes first, then pipette the volume of 50ul from each tube to microplate well,

1800 pg/ml	Standard No.1	300µL Original Standard + 150 µL Standard diluents
1200 pg/ml	Standard No.2	300µL Standard No.1 + 150 µL Standard diluents
600 pg/ml	Standard No.3	150µL Standard No.2 + 150 µL Standard diluent
300 pg/ml	Standard No.4	150 µL Standard No.3 + 150 µL Standard diluent
150 pg/ml	Standard No.5	150 µL Standard No.4 + 150 µL Standard diluent

Preparation procedure steps of standard were summarized below



2.10 Samples and Reagents Preparation

Stock solutions were prepared based on the procedure of the manufactured kit. All reagents were prepared freshly at room temperature before used.:

1) In the Micro-Elisa strip plate, leave a well empty as blank control. In sample wells, 40µl Sample dilution buffer and 10µl sample are added (dilution factor is 5). Samples should be loaded onto the bottom without touching the well wall. Mix well with gentle shaking.
2) Incubation: incubate 30 min at 37℃ after sealed with Closure plate membrane.
3) Dilution: dilute the concentrated washing buffer with distilled water (30 times for 96T and 20 times for 48T).
4) Washing: carefully peel off Closure plate membrane, aspirate and refill with the wash solution. Discard the wash solution after resting for 30 seconds. Repeat the washing procedure for 5 times.
5) Add 50 µl HRP-Conjugate reagent to each well except the blank control well.
6) Incubation as described in Step 3.
7) Washing as described in Step 5.
8) Coloring: Add 50 µl Chromogen Solution A and 50 µl Chromogen Solution B to each well, mix with gently shaking and incubate at 37℃ for 15 minutes. Please avoid light during coloring.
9) Termination: add 50 µl stop solution to each well to terminate the reaction. The color in the well should change from blue to yellow.
10) Read absorbance O.D. at 450nm using a Microtiter Plate Reader. The OD value of the blank control well is set as zero. Assay should be carried out within 15 minutes after adding stop solution.

2.11 Calculation of Results

Known concentrations of Human chemerin Standard and its corresponding reading OD is plotted on the log scale (x-axis) and the log scale (y-axis) respectively. The concentration of Human chemerin in sample is determined by plotting the sample's O.D. on the Y-axis. The original concentration is calculated by multiplying the dilution factor The original concentration is calculated by multiplying the dilution factor.

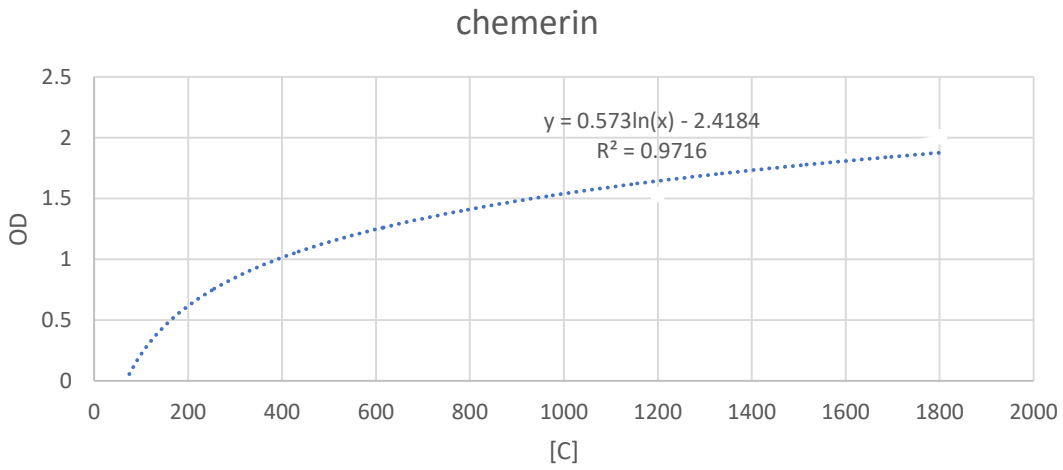


Figure 2.1: Standard Curve of the Human chemerin concentration .

2.11.1 Measurement of Nesfatin-1 levels in Human :

Principle:

This ELISA kit uses Sandwich-ELISA as the method. The Micro ELISA strip plate provided in this kit has been pre-coated with an antibody specific to Nesfatin-1 Standards or samples that are added to the appropriate Micro elisa strip plate wells and combined to the specific antibody. Then a Horseradish Peroxidase (HRP)-conjugated antibody specific for Nesfatin-1 is added to

each Micro Elisa strip plate well and incubated. Free components are washed away. The TMB substrate solution is added to each well. Only those wells that

contain Nesfatin-1 and HRP conjugated Nesfatin-1 antibody will appear blue in color and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of Nesfatin-1. The concentration of Nesfatin-1 in the samples was calculated by comparing the OD of the samples to the standard curve.

Reagents :

The Measurement of Nesfatin-1 levels method reagents are below:

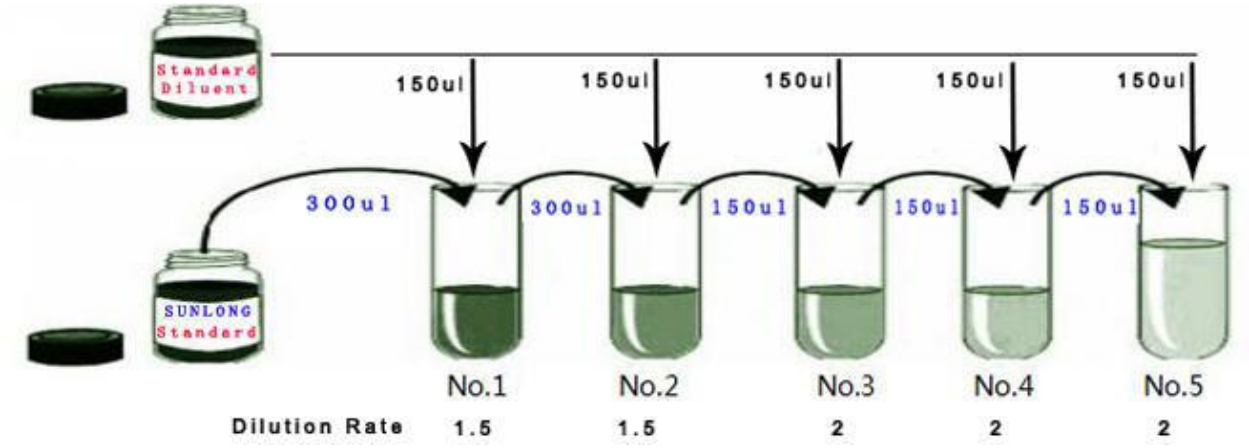
Materials provided with the kit	96 determinations	Storage
User manual	1	R.T.
Closure plate membrane	2	R.T.
Sealed bags	1	R.T.
Microelisa stripplate	1	2-8℃
Standard：2700 pg/ml	0.5ml×1 bottle	2-8℃
Standard diluent	1.5ml×1 bottle	2-8℃
HRP-Conjugate reagent	6ml×1 bottle	2-8℃
Sample diluent	6ml×1 bottle	2-8℃
Chromogen Solution A	6ml×1 bottle	2-8℃
Chromogen Solution B	6ml×1 bottle	2-8℃
Stop Solution	6ml×1 bottle	2-8℃
wash solution	20ml (30X)×1bottle	2-8℃

Preparing of Standard Curve:

Dilute the standard by small tubes first, then pipette the volume of 50ul from each tube to microplate well,

360 pg/ml	Standard No.1	300µl Original Standard + 150µl Standard diluents
240 pg/ml	Standard No.2	300µl Standard No.1 + 150µl Standard diluents
120 pg/ml	Standard No.3	150µl Standard No.2 + 150µl Standard diluent
60 pg/ml	Standard No.4	150µl Standard No.3 + 150µl Standard diluent
30 pg/ml	Standard No.5	150µl Standard No.4 + 150µl Standard diluent

Preparation procedure steps of standard were summarized below:



2.11.2 Samples and Reagents Preparation

Stock solutions were prepared based on the procedure of the manufactured kit. All reagents were prepared freshly at room temperature before used:

1- In the Micro-Elisa strip plate, leave a well empty as blank control. In sample wells, 40µl Sample dilution buffer and 10µl sample are added (dilution factor is 5). Samples should be loaded onto the bottom without touching the well wall. Mix well with gentle shaking.
2- Incubation: incubate 30 min at 37℃ after sealed with Closure plate membrane.
3- Dilution: dilute the concentrated washing buffer with distilled water (30 times for 96T and 20 times for 48T).
4- Washing: carefully peel off Closure plate membrane, aspirate and refill with the wash solution. Discard the wash solution after resting for 30 seconds. Repeat the washing procedure for 5 times.
5- Add 50 µl HRP-Conjugate reagent to each well except the blank control well.
6- Incubation as described in Step 3.
7- Washing as described in Step 5.
8- Coloring: Add 50 µl Chromogen Solution A and 50 µl Chromogen Solution B to each well, mix with gently shaking and incubate at 37℃ for 15 minutes. Please avoid light during coloring.
9- Termination: add 50 µl stop solution to each well to terminate the reaction. The color in the well should be change from blue to yellow.
10- Read absorbance O.D. at 450nm using a Microtiter Plate Reader. The OD value of the blank control well is set as zero. Assay should be carried out within 15 minutes after adding stop solution.

2.12 Calculation of Results

Known concentrations of Human **Nesfatin-1** Standard and its corresponding reading OD is plotted on the log scale (x-axis) and the log scale (y-axis) respectively. The concentration of Human **Nesfatin-1** in sample is determined by plotting the sample's O.D. on the Y-axis. The original concentration is calculated by multiplying the dilution factor The original concentration is calculated by multiplying the dilution factor.

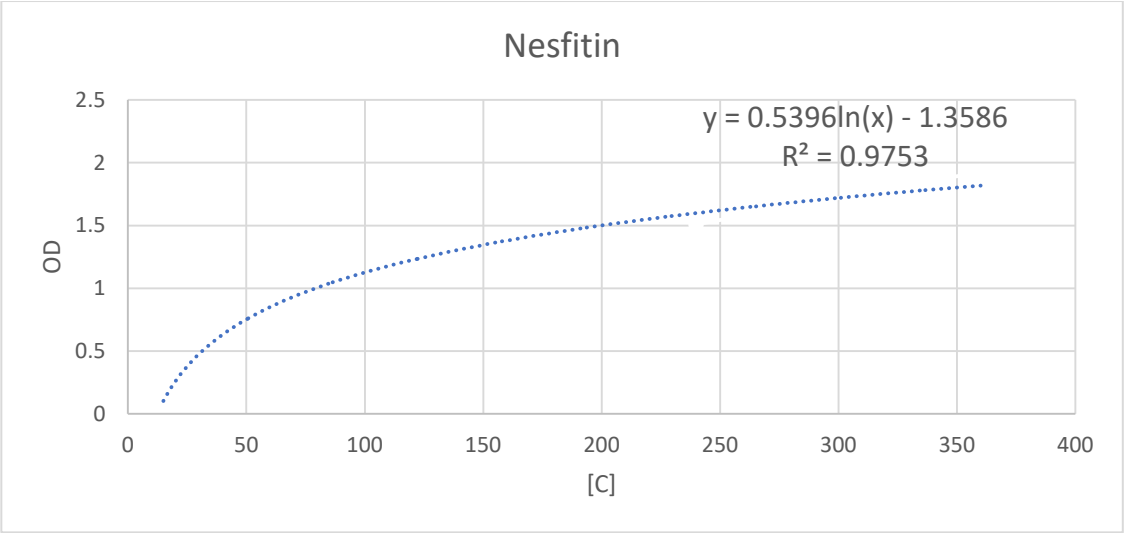


Figure 2.2: Standard Curve of the Human Nesfitin concentration.

2.12.1 Measurement of Lipid profile levels in Human serum by using Spectrophotometric Technique:

2.12.1.2 Method for Quantitation Serum total cholesterol level:

Enzymatic colorimetric method was used to measure concentration of serum total cholesterol,This method for the measurement of total cholesterol in serum involves the use of three enzymes: cholesterol esterase (CE), cholesterol oxidase (CO) and peroxidase (POD). In the presence of the former, the mixture of phenol and 4-aminoantipyrine (4-AA) are condensed by hydrogen peroxide to form a quinoneimine dye proportional to the concentration of cholesterol in the sample.

The absorbance of total cholesterol was measured at the wavelength 500 nm. Reference values for serum adults 50-199 mg/dL (92).

2.12.1.2 Method for Quantitation Serum Triglyceride level:

Enzymatic colorimetric method was used to measure concentration of serum triglyceride, The method was based on the enzymatic hydrolysis of serum or plasma triglyceride to glycerol and free fatty acids (FFA) by lipoprotein lipase (LPL). The glycerol is phosphorylated by adenosin triphosphate (ATP) in the presence of glycerolkinase (GK) to form glycerol-3-phosphate (G-3-P) and adenosine diphosphate (ADP). G-3-P is oxidized by glycerophosphate oxidase (GPO) to form dihydroxyacetone phosphate (DHAP) and hydrogen peroxide. A red chromogen is produced by the peroxidase (POD) catalyzed coupling of 4-aminoantipyrine (4-AA) and phenol with hydrogen peroxide (H2O2), proportional to the concentration of triglyceride in the sample. The absorbance of Triglyceride was measured at the wavelength 500 nm. Reference values for serum adults 0-149 mg/dL (93).

2.12.1.3 Method for Quantitation Serum High Density Lipoprotein level:

Enzymatic colorimetric test was used to measure concentration of serum high density lipoprotein. This technique used a separation method based on the selective precipitation of apoliprotein B-containing lipoproteins (VLDL, LDL and (a)Lpa) by phosphotungstic acid/MgCl2, sedimentation of the precipitant by centrifugation, and subsequent enzymatic analysis of high density lipoproteins (HDL) as residual cholesterol remaining in the clear supernatant. The absorbance of HDL was measured at the wavelength 500 nm. Reference values for serum adults 40-60 mg/dL (94).

2.12.1.4: Method for Quantitation Serum Low Density Lipoprotein level:

Enzymatic colorimetric test was used to measure concentration of serum low density lipoprotein. This technique used a separation method based on the specific precipitation of low-density lipoproteins (LDL) by polyvinyl sulfate in whole serum, sedimentation of the precipitant by centrifugation, and subsequent test as residual cholesterol of the rest of lipoproteins (VLDL+ HDL) remaining in the clear supernatant. LDL-cholesterol was calculated by substracting the supernatant cholesterol fractions from the total cholesterol of the sample. The absorbance of LDL was measured at the wavelength 500 nm. Reference values for serum adults were 0-100 mg/dL optimal, 100-129 mg/dl above optimal, 130-159 mg/dl borderline high (95).

2.13: Statistical Analysis:

Information from the questionnaire and all test results from patients and control samples were entered a data sheet. The analysis was carried out using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL) version 28.

Categorical variables were presented as numbers and percentages, Scale variables were presented by mean ± 2standard deviation (mean±SD) for normal data while, non-normal data, continuous variables were presented by interquartile range (IQR) and median. For abnormal distribution, the univariate analysis was performed using an independent Mann–Whitney U test for continuous variables and unpaired Student’s t-tests for normally distributed continuous variables which were used to establish the correlations. Biomarkers were compared using Spearman rank test to evaluate the relationship with the case study.

The optimal threshold with high specificity and sensitivity for Arrhythmia SVT cases were detected using receiver operating characteristic (ROC) analysis. It was found out that all the values of P were two-sided, and a $P < 0.05$ was considered to be statistically significant.

Chapter Three

Results & discussion

3. Results

Adipokines are produced from adipose tissues, which are considered endocrine organs involved in cardiovascular function causing electrophysiological effects such as ionic profiles, contractility in the atrium, and change in morphology of action potential. Also, adipokines have cardioprotective effects; however, it has been reported that the adipokines which involved in predisposing to cardiotoxicity are chemerin, and Nesfitin (96). Therefore, this study was amis to investigate and focuses on the basic role of few adipokines associated with Atrial Fibrillation.

3.1 Demographic and clinical characteristics

The clinical demographic characteristics and laboratory parameters of patients group were summarized in tables (3.1) & (3.2). The mean age of participants which was within the age group of (16-62) years old. Gender distribution among the studied groups were: 75% male, 25% female for patients' group. Mean of heart rate was increased vigorously (188 bpm) compared to control group. Mostly of participants were no having severe medical history such as diabetes mellitus, hypertension and Endocrine disorder.

Table 3.1: Baseline characteristics and Demographic Descriptive of the study population in cases of SVT patients compared to control group (n= 90)

Variable	Groups	Patients Groups	Healthy Control Group
Baseline characteristics	Age range Years Old	16-62	17-57
	Gender (Female/ Male) No.	15/45	10/20
	BMI (Kg/m^2)	30.2	29.9
	Smoking state (Yes/No) No.	6/54	/

Medical history	Mean of Heart rate beat/min	188	79.4
	HT (Yes/No) No.	10/50	/
	DM (Yes/No) No.	12/48	/
	Endocrine disorder(Yes/No)	16/44	/
Results are presented as n= number of subjects and percentage. BMI: Body Mass Index , HT : Hypertension , DM : diabetes, HF: Herat failure			

Table 3.2: Baseline characteristics of the study population in cases of SVT patients compared to control group (n= 90)

Biochemical Investigations				
Parameter	Patients Groups		Healthy Control Group	
	Mean ±2SD	Median (Mini-Max)	Mean ±2SD	Median (Mini-Max)
Nesfitin	134.8±19.5	130.1(86.6-183.7)	151.8±27.6	143.6 (108.9-207.2)
Chemerin	734.1±95.04	714.7 (487.6-986.9)	673.8±81.9	668.8 (538.6-907.6)
Chol	162.7±56.5	136 (105.9-287)	127.2±9.3	129.0 (106.0-136.0)
TG	142.4±58.01	120 (84-384.6)	109.7±16.6	106 (83.0-138.0)
HDL	58.3±10.1	55.5 (43-77)	50.5±4.25	51 (41.0-58.0)
LDL	75.9 ±42.7	61.9 (26.3-183.6)	54.8±10.9	58.5 (35.0-69.8)
VLDL	28.5±11.6	24 (16.8-76.92)	21.9±3.3	21.2 (16.6-27.6)
Results are presented as mean ± SD, p<0.05 considered significantly different, [S]= Significant, [NS]= Non significant				

T- test

3.2 Examination the distribution of data in the studied groups:

3.2.1 Distribution of serum level of Nesfitin & Chemerin:

A box plot was used to visually show the distribution of data through displaying the data quartiles and averages. Figure (3.1) demonstrated a across distribution of serum level of Nesfitin & Chemerin in SVT patients group and healthy control

group. Throughout the results, the quartiles and range levels of Nesfitin was decreased markedly in arrhythmia patients, while Chemerin was estimated to have great variability in patients compared to control. The range levels in patients groups were (86.6-183.7) & (487.6-986.9) ng/ml respectively.

Adipokines are cardiovascular disease (CVD) mediators or biomarkers that affect the heart as well as blood vessels, by increasing the cardiac contractility and action potential duration, which result in the extent of left ventricular and atrial remodeling. Adipokines play a significant role in the development and progress of atrial fibrillation. The pathophysiological role in atrial fibrillation by causing cardiac hypertrophy is manifested by increasing the cardiac contractility and action potential duration, atrial fibrosis, electrical and structural remodeling of atrial tissue (97).

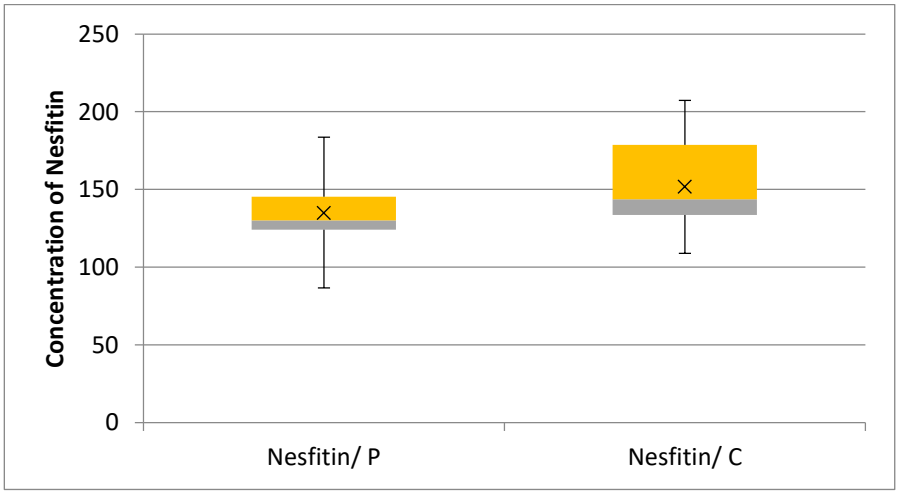
Zhu et al. (2021) also found that serum adiponectin (chemerin) was associated with the markers of cardiac autonomic, inflammation as well as cardiac remodeling. Moreover, subsequent multivariate analysis showed a significant independent link between elevated adiponectin levels and atrial fibrillation in the overall participants (98).In addition, Kourliouros et al. also explained that a greater inflammation environment could provide a substrate for the development of arrhythmia (99).

Additionally, Kusayama et al. study had revealed that inflammation has been associated with the presence of paroxysmal atrial fibrillation (100). In atrial fibrillation-related structural remodeling, the predominant pathologic abnormality is the atrial fibrosis and also clinical significance has a degree of fibrosis. Epicardial fat is a metabolically active tissue that generates a variety of bioactive molecules including TNF-α and adiponectin which can be the key mediators of atrial fibrosis as well as structural remodeling (99).

Adiponectin influences the functioning of the heart through central nervous system when adiponectin enters through the blood–brain barrier. Adipocytokine imbalance including a lower level of adiponectin (Nesfitin) which results in the development of hyperinsulinemia, dyslipidemia, endothelial dysfunction, fibrosis, abdominal obesity, arterial hypertension, impaired glucose tolerance, and atrial fibrillation (101).

Similarly, Linberg and Karas studies have shown a connection between quite high levels of chemerin and heart failure, coronary heart disease, and even mortality (102).

On the other hand, Endogenously expressed NF-1 was recently identified in the heart of mammals, suggesting that NF-1 may be an endogenous modulator of cardiac performance. Indeed, NF-1 has been shown to induce negative inotropism and lusitropism in vitro (103). Nesfitin attenuates cardiac performance as indicated by decreases in left ventricular (an index of myocardial contractility) and left ventricular fractional shortening in perfused hearts (104). However, the mechanisms underlying these effects of NF-1 in cardiomyocytes remain unknown.



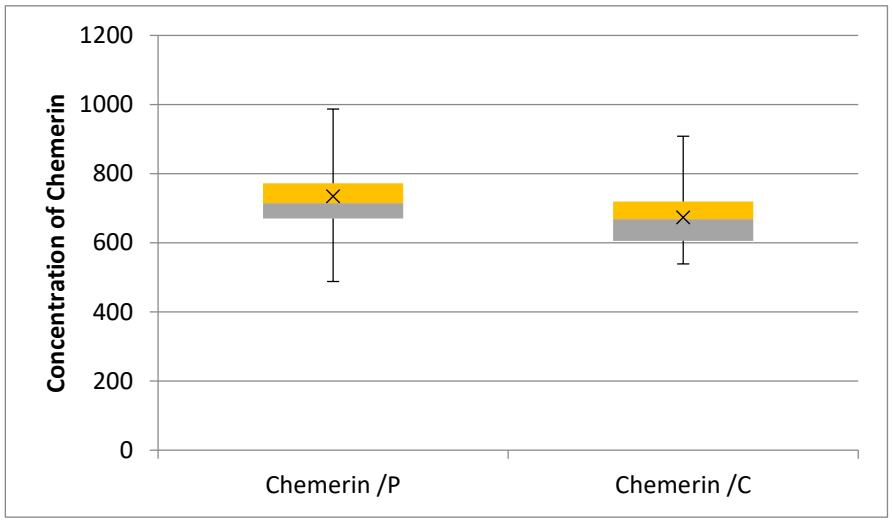


Figure 3.1: Boxplot of the Distribution of serum level of Nesfitin & Chemerin in SVT with risk factor patients & Healthy control group.

3.2.2 Distribution of serum level of Lipid profile panel :

The distribution of serum lipid profile levels in SVT with risk factor patients group compared to the healthy group was also examined which also indicated abnormal distribution as shown in figure (3.2).

Mostly, patients with SVT with risk factor were shown an increased level of the measured lipid profile. Increased serum cholesterol levels can be explained by increased systemic inflammation and oxidative stress, which may increase oxidized cholesterol level with an increase in LDL cholesterol level. Many other factors has been reported to affect the cholesterol levels such: body weight-for height measures, systolic and diastolic blood pressures, and serum total cholesterol, LDL-cholesterol, homocysteine, creatinine, and parathyroid hormone concentrations (105).

Serum triglyceride is one of the most valuable lipid types altered in heart diseases, mostly occurs in early stages due to both abnormal production and reduced

catabolism of triglycerides. The alteration of the catabolism of triglycerides occurs due to the inactivation of the lipoprotein lipase (LPL).

Increased apolipoprotein C-III/C-II ratio precipitates the inactivation of the LPL, since apolipoprotein C-III is an inactivator for LPL, whereas apolipoprotein C-II is an activator for LPL (106).

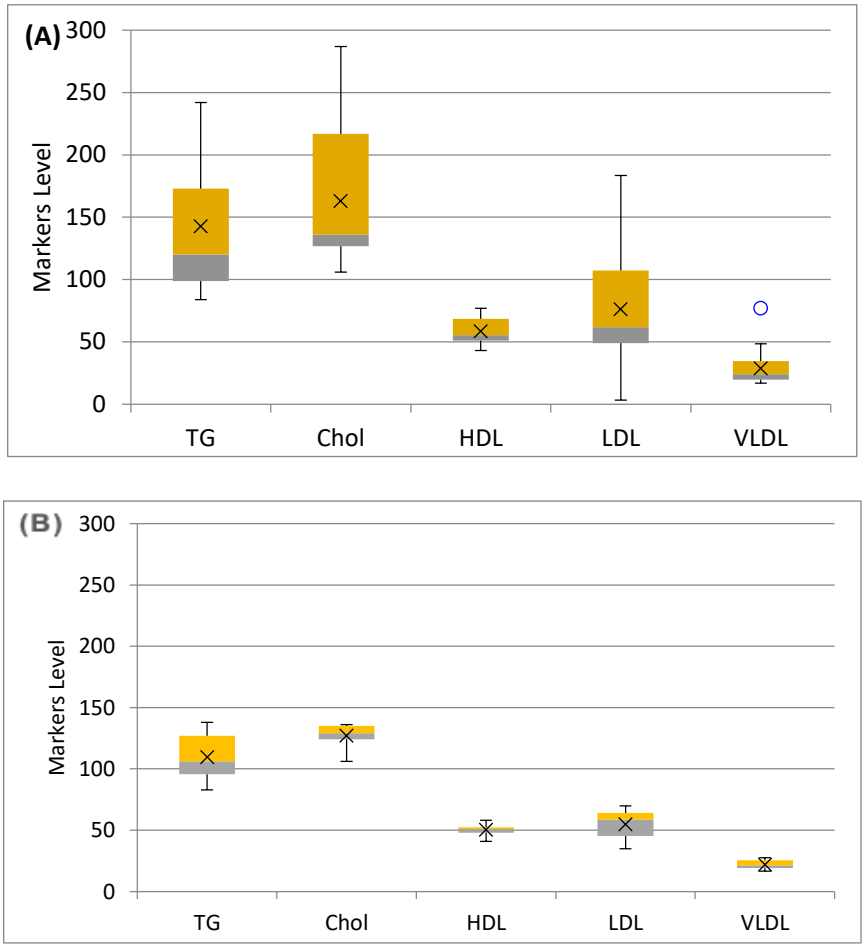


Figure 3.2: Boxplot of the Distribution of serum lipid profile panel in: (A) SVT with risk factor patients; (B) Healthy control group

3.3 Study the effect of case groups

Serum nesfatin-1 level was measured in three groups consisting of SVT arrhythmia certain etiology patients; SVT un-certain etiology patients and Healthy control group. According to the results, the mean level of serum nesfatin-1 was significantly higher in normal subjects than in both certain & un-certain SVT

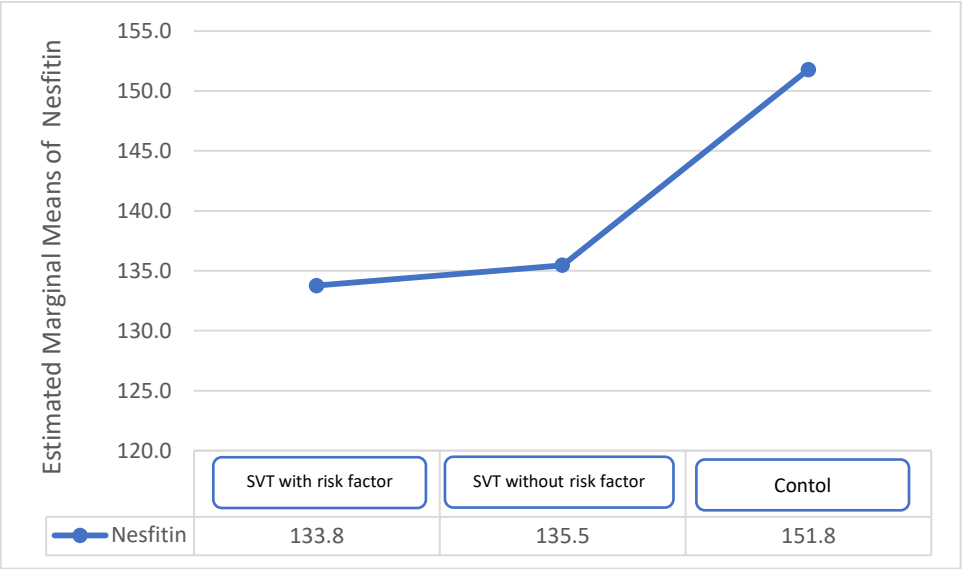
etiology groups. The mean serum nesfatin-1 level was differ between the studied groups (133.7±19.8; 135.46±19.56; 151.7875±27.55 ng/mL respectively, P = 0.014).

Based on the previously reported results about the role of Chemerin a good proposed and predictive marker of cardiovascular risk, since its circulating levels correlate with the severity of disease, and patients with dilated cardiomyopathy and with acute myocardial infarction since it has higher concentrations of circulating chemerin. Estimation plot of determination serum level of Chemerin indicted a massive increased level in SVT without risk factor patients compared the other groups. the serum concentrations of Chemerin (mean=760.8± 107.80; P<0.001) were significantly higher in such patients as compared with those in the SVT with risk factor and control group (mean=689.7± 42.5; 673.8± 81.9) as presented in Table (3.3) & Figure (3.3). Many studies were examined the role of adipokines (chemerin & Nesfitin) in different cardiac event, Gao et al found that the mRNA and protein expression levels of chemerin were significantly upregulated in epicardial adipose tissue from patients with CAD and the severity of coronary atherosclerosis was positively related to the chemerin expression . In addition, Rodrıguez-Penas et al reported that chemerin was regulated by metabolic and inflammatory mediators at the cardiac level, and it could induce apoptosis and inhibit protein kinase B phosphorylation in cardiomyocytes (107).

Furthermore, chemerin promoted adhesion of macrophages to vascular cell adhesion molecule 1 and fibronectin by clustering VLA-4 (a4b1) and VLA-5 (a5b1), thereby contributing to inflammation Which may reflect the extent of coronary atherosclerosis. Zhang et al indicated that serum chemerin concentration was increased in patients with dilated cardiomyopathy and chemerin was

markedly associated with inflammatory response and left ventricle dysfunction (108). The massive increased serum chemerin levels in group patients of uncertain etiology of arrhythmia was a significant predictor for the SVT cases.

On the other hand, L-type voltage-gated Ca^{2+} channels (VGCC) are voltage-dependent channels that open in response to membrane depolarization, permitting entry of Ca^{2+} into the cell (109). The depolarizing current through L-type VGCC contributes to the plateau phase of the cardiac action potential as well as to pacemaker activity in nodal cells (110). The influx of Ca^{2+} subsequently triggers the release of intracellular Ca^{2+} stores from the sarcoplasmic reticulum, and the ensuing intracellular Ca^{2+} transient results in the activation of the myofilaments, allowing cell contraction (111). Additionally, L-type VGCC can affect other cellular processes modulated by intracellular Ca^{2+}, including gene expression and excitation-secretion coupling (112). Therefore, alterations in density or function of L-type VGCC have been implicated in a variety of cardiovascular diseases, including atrial fibrillation, heart failure, and ischemic heart disease (113). Altering the properties of the VGCC could have detrimental effects on cardiac electrical and contractile functions (114). Importantly, these channels are modulated by a variety of hormones, neurotransmitters, and cytokines, operating via G-protein coupled receptors and second messengers (115), and thereby profoundly affecting the functions of target tissue. Nonetheless, there is little information available on the underlying mechanism of the effect of NF-1 on cardiac L-type Ca^{2+} channels.



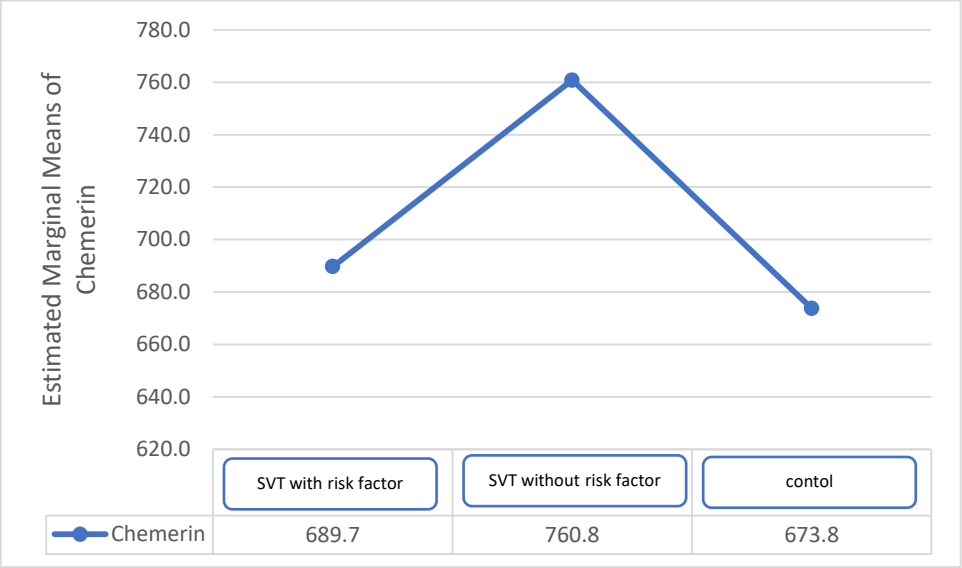


Figure 3.3: Estimation plot of Determination serum level of Nesfitin & Chemerin in: SVT with risk factor etiology patients; SVT without risk factor etiology patients and Healthy control group

Table 3.3 : Multiple Comparisons of Dependent Variable and Least Significant Difference-Post Hoc Test for Nesfitin & Chemerin level with Study groups

Biochemical parameters	(N=90)			P value
	SVT with risk factor N= 30	SVT without risk factor N= 30	Control N= 30	
Nesfitin	133.7±19.8	135.46±19.56	151.78±27.55	0.014[S]

Chemerin	689.7± 42.5	760.8± 107.80	673.8± 81.9	0.001[S]
Results are presented as mean ± SD, p<0.05 considered significantly different, [S]= Significant, [NS]= Non significant,				

ANOVA test

3.3.1 Study the effect of Body Mass Index on the SVT patients groups

Obesity is considered as a risk factor for arrathmias development. The Framingham Heart Study demonstrated that greater pericardial fat deposition is associated with increased risk of disease incidence (116).

The study was also included the examination of independent variables (BMI) on the measured biomarkers namely Nesfitin & Chemerin. Patients were divided into three groups based on the standard groups of BMI (Normal; over wight; and obese).

Table (3.4) show the comparison of biomarkers levels between patients groups which were statistically not showing any significant differences in Nesfitin & Chemerin, p value > 0.05

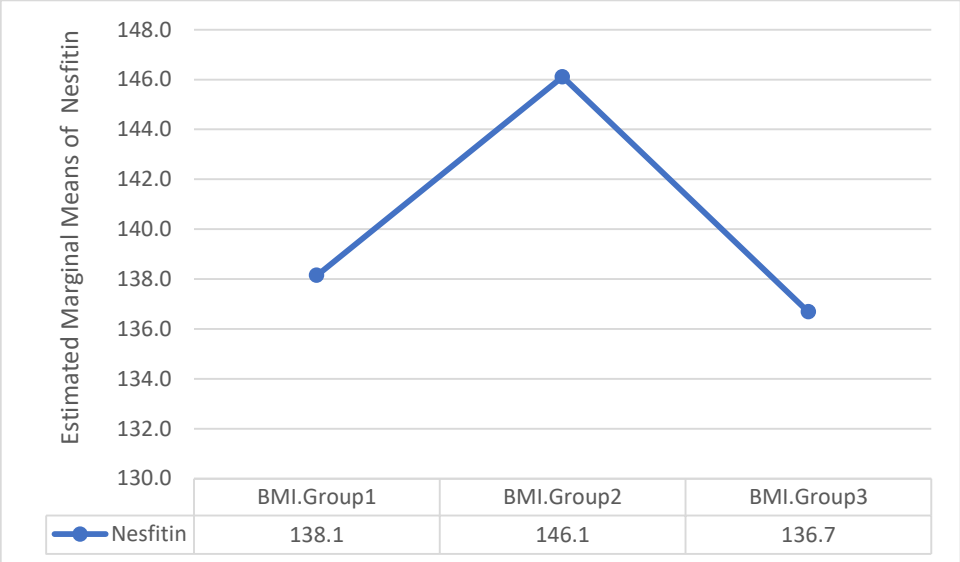
According to the previous studies, it might be hypothesized that the link between arrythmias and adiponectin could be age as well as gender-dependent. In addition, the most important pathogenetic factors are autonomic, atrial remodeling, and inflammation for atrial fibrillation (117).

multivariate analysis of BMI Subgroup showed a significant association between higher adiponectin levels and arrythmias. A sort of conflicting results may be explained by the potential influences of sex, age, or comorbidities. Chemerin is believed to be a link between obesity and inflammation, its levels are particularly high in very obese subjects, severe obesity (118). It is secreted as an inactive precursor activated by serine proteases associated with cascades of coagulation, fibrinolysis and inflammation (119).

Since nesfatin-1 has described as anorexigenic peptide exerting a robust reduction of body weight, However, many studies indicated a negative association of circulating nesfatin-1 and body mass index (BMI) (120) (121).

Previously, study was indicated that Obese population showed lower serum nesfatin-1 than control subjects (122) (18). This finding suggests that nesfatin-1 serves as a link between obesity and AF. However, the exact mechanism involved in this phenomenon warrants further investigation (123).

lipid accumulation was reduced by nesfatin-1 in cultured hepatocytes in vitro, probably because of a decrease of lipogenesis-relevant genes such as peroxisome proliferator-activated receptor-γ and sterol-regulatory element-binding protein 1 and enzymes including fatty acid synthase and glycerol-3-phosphatase acyltransferase, whereas β-oxidation-related genes were increased (124). the mechanisms of endocrine and metabolic effects of nesfatin-1 have not been known well, this role needs further investigated (121).



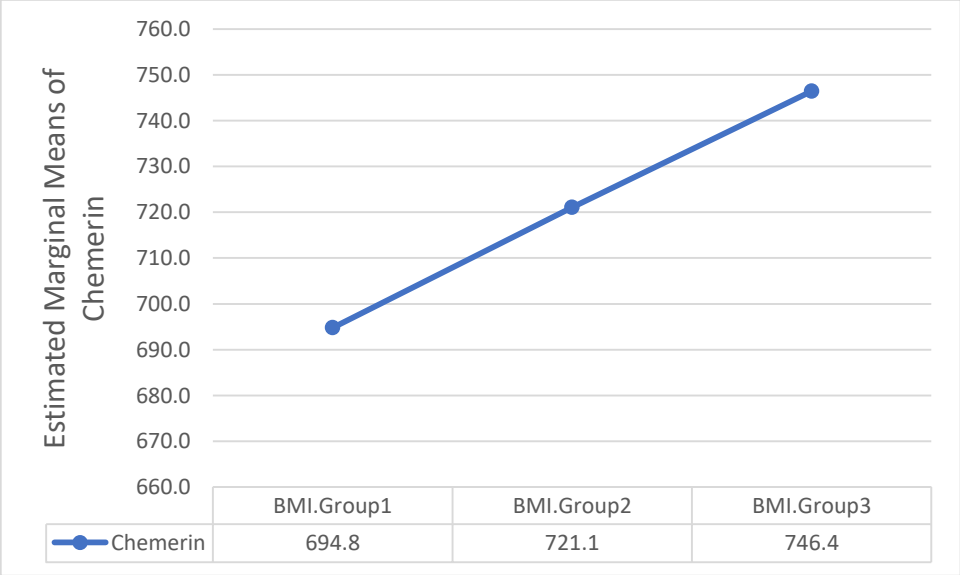


Figure 3.4: Estimation plot of Determination serum level of Nesfitin & Chemerin in different BMI groups

Table 3.4: Multiple Comparisons of Dependent Variable and Least Significant Difference-Post Hoc Test for Nesfitin & Chemerin level based on BMI

Biochemical Parameters	(N=60)			P value
	Group 1 (NormalWeight)	Group 2 (Over Weight)	Group 3 (Obese)	
Nesfitin	138.1±19.03	146.1±14.59	136.7± 23.28	0.90[NS]
Chemerin	694.8±97.00	721.1 ±87.97	746.4±96.25	0.14[NS]
Results are presented as mean ± SD, p<0.05 considered significantly different, [S]= Significant, [NS]= Non significant				

ANOVA test

3.3.2 Study the association of Adipokines and patients' groups

Binary logistic regression was performed and forward logistic regression was adopted to analyze the results. It was found that chemerin in certain and uncertain etiology SVT patient (OR: 1.008 and 1.018; 95% CI: (0.998 - 1.018), (1.008 - 1.028) respectively, were independent risk factors.

while Nesfitin (OR: 0.960 and 0.946, 95% CI: (0.929 - 0.992), (0.913 - 0.980) were independent protective factors for arrhythmia SVT patient, as shown in Table (3.5)

Table 3.5: Estimation the associated of analyzed factors (Nesfitin & Chemerin) in SVT Patients Compared to control group

Variables	Case study	OR (95% CI)	P value
Nesfitin	SVT with risk factor	0.960 (0.929 - 0.992)	0.014 [S]
	SVT without risk factor	0.946 (0.913 - 0.980)	0.002 [S]
	Control	1[a]	-
Results are presented as numbers and percentage, p<0.05 considered significantly different, [S]; Significant, [NS]; Non significant, OR: Odds Ratio, CI; Confidence IntervalIL			

3.4 Study the correlation of Adipokines and patients' groups

Considering the important role of the measured biomarkers in the, the spearman rank test analysis of SVT patient was used to analyze the response relationship between parameters (126). As a risk factor of arrhythmia cases indicator, serum TG was highly significant positively related to high levels of VLDL (all P < 0.001). Similarly, serum Cholestreol values were correlated with LDL levels. The relationship between the parameters and study cases was presented in Figure (3.5)

It has been reporting by Pearson correlation analysis that there was a positive association of serum concentrations with diastolic blood pressure, body mass index (BMI), systolic blood pressure, triglycerides, low-density lipoprotein cholesterol (LDL-C), and creatinine. As a result, they showed an association of serum chemerin concentration with atrial remodeling (127).

Also, circulating levels was correlate positively with the severity dilated cardiomyopathy. Because of its chemotactic effects mediated reduction in NO production and negative effects on plasma lipids, chemerin is linked to progression of atherosclerosis (128). Low serum levels of cardio-protective adipokines(nesfitin) or increased levels of pro-inflammatory adipokines (chemerin) might be useful biomarkers of different cardiovascular diseases. Despite the vigorous research in the field of adipokines, it may take some more time to very clearly establish the role of each adipokine in health and disease conditions (129).

Kaur et al (130). Validated that recombinant chemerin significantly induced tube formation in endothelial cells. Moreover, associations with high-sensitivity C-reactive protein (hsCRP), white blood cell count, markers of endothelial activation intercellular cell adhesion molecule-1 (ICAM-1) and E-selectin suggests a link of chemerin not only with obesity and inflammation but also to endothelial activation markers (131).

	Nesfitin	Chemerin	TG	Cholestreol	HDL	LDL	VLDL	Urea	Creatinin	BMI
Nesfitin	1									
Chemerin										
TG										
Cholestreol										
HDL										
LDL			.**							
VLDL										
Urea										
Creatinin										
BMI										

Figure 3.5: Heatmap of the spearman rank test analysis of SVT patient. white boxes indicate a lack of correlation (p>0.05) while in coloured boxes were reported statistically significant direct and indirect correlations, respectively. The intensity of the colour indicates the following relation: green (r=0.4); blue (r=0.5) light blue (r=0.7); orang(r=0.9).

3.5 Receiver operating curve (ROC) curve of serum Adepokines levels to diagnosis of SVT cases

Results of the receiver operating curve (ROC) curve and AUC analysis for the the Nesfitin and chemerin besides their ratio as possible diagnostic parameters. only chemerin and chemerin/ Nesfitin ratio were shown a good diagnostic performance for prediction arrhythmia Patients compared to control group, data are presented in Table (3.6).

For chemerin levels: (sensitivity = 0.65.%, specificity = 0.67%) at a level = 690.1 , while chemerin/ Nesfitin ratio levels: (sensitivity = 0.92%, specificity = 0.67%)

at a level = 4.61 and the calculated accuracy was (83.33%). Acorrdingly, the distribution of patients using chemerin/ Nesfitin ratio cutoff values was presented in Table (3.7).

The p-values of the AUC were <0.05 and statistically significant. Youden's J statistics of the parameters in Figures (3.6) & (3.7) confirm these results.

Table 3.6: AUC, optimal threshold, Sensitivity and specificity of proposed marker obtained by the ROC curves for prediction of arrhythmia patients

Test Variable	Chemerin	Chemerin/ Nesfitin
AUP	0.7	0.82
Sensitivity %	65%	92%
Specificity %	67%	67%
Youden index	0.313	0.584
Cut-off points	690.1	4.61
CI (95%)	0.55 – 0.81	0.70 – 0.93
PPV	79.48%	84.62%
NPV	48.48	80%
Accuracy	65.27%	83.33%
P value	0.012	<0.001

AUP: Area under pick, CI: con confidence interval, PPV : positive protective value , NPP: Negative protective value

Chemerin/ Nesfitin ratio cutoff values	Patients	Control
<4.6066	55	10
>4.6066	5	20
Total	60	30

Table 3.7. Distribution of patients according to the CNR cutoff values in the studied groups

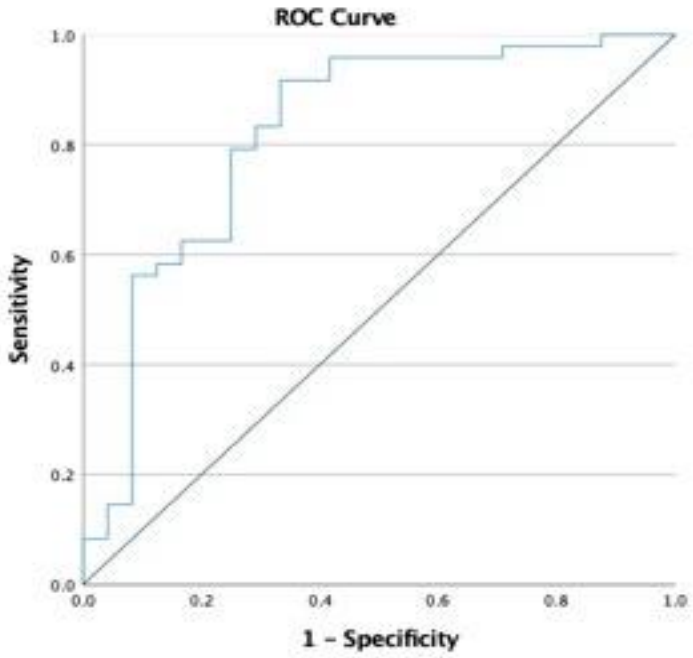


Figure 3.6: ROC curves for, chemerin/ Nesfitin ratio in arrhythmia patients to analyse the optimal diagnostic points for predicting SVT cases compared to control group, The area under ROC curve:0.82; 95%CI (0.70 – 0.93); p value <0.05

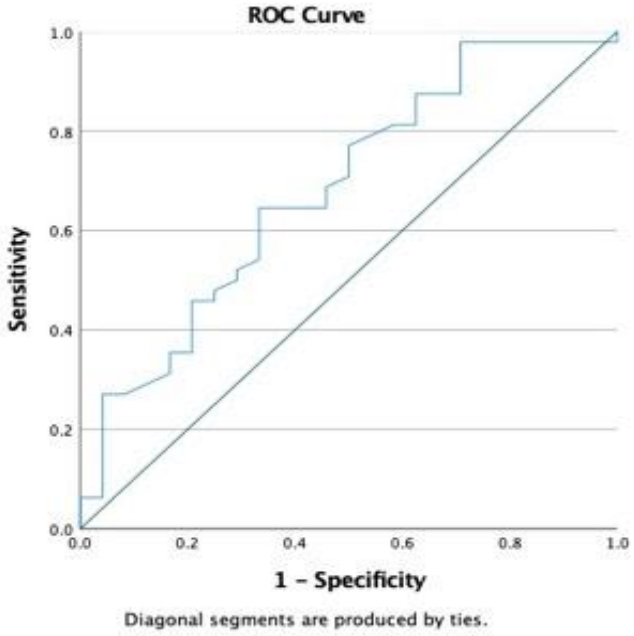


Figure 3.7: ROC curves for chemerin in arrhythmia patients to analyse the optimal diagnostic points for predicting SVT cases compared to control group, The area under ROC curve:0.7; 95%CI (0.55 – 0.81); p value <0.05

Furthermore, the analysis of the optimal diagnostic points for predicting SVT without risk factor etiology of SVT cases compared to SVT with risk factor group was performed.

Results were indicated that chemerin was demonstrated the most interesting prediction about SVT without risk factor etiology of SVT cases. The optimal threshold and diagnostic performance was presented in table (3.8) ROC curves were presented in figures (3.8), (3.9) and (3.10). Accordingly, the ddistribution of patients using chemerin cut-off values was presented in table (3.9).

Table 3.8: AUC, optimal threshold, Sensitivity and specificity of proposed marker obtained by the ROC curves for prediction uncertain etiology of SVT cases compared to SVT with risk factor group.

Test Variable	Chemerin	Chemerin/ Nesfitin	Nesfitin
AUP	0.74	0.64	0.6
Sensitivity %	0.6	0.4	0.4
Specificity %	0.889	0.889	0.778
Youden index	0.489	0.289	0.178
Cut-off points	733.55	5.9337	139.25
CI (95%)	0.597 - 0.875	0.482 – 0.807	0.396 - 0.734
PPV	57.14%	47.05%	43.75%
NPV	90%	85.71%	54.16%
Accuracy	70.83%	58.33%	75%
P value	<0.001	0.033	0.206

AUP: Area under pick, CI: con confidence interval, PPV : positive protective value , NPP: Negative protective value

Table 3.9: Distribution of patients according to the Chemerin cut-off value in subgroups

Chemerin	SVT with risk factor	SVT without risk factor
<733.55	27	12
>733.55	3	18
Total	30	30

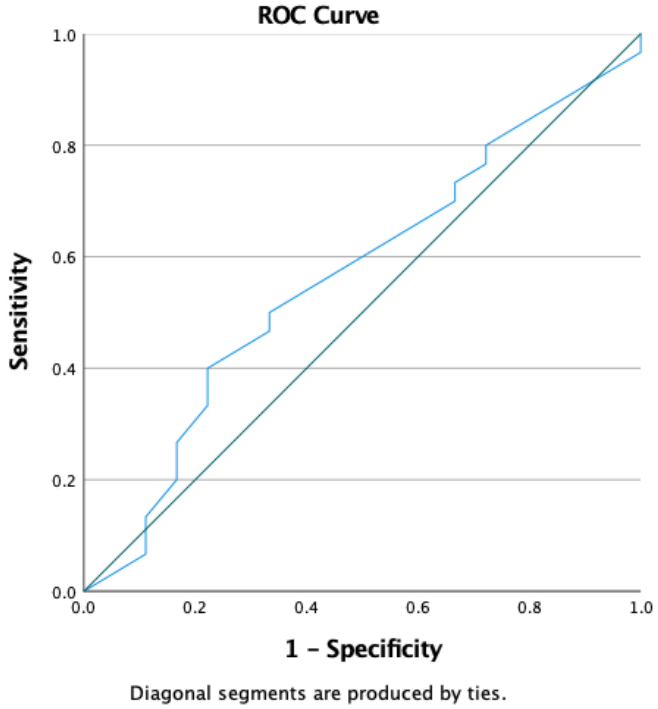


***Figure 3.8: ROC curves for Nesfitin in arrhythmia patients to analyse the optimal diagnostic points for predicting of* SVT without risk factor cases compared to other group.**

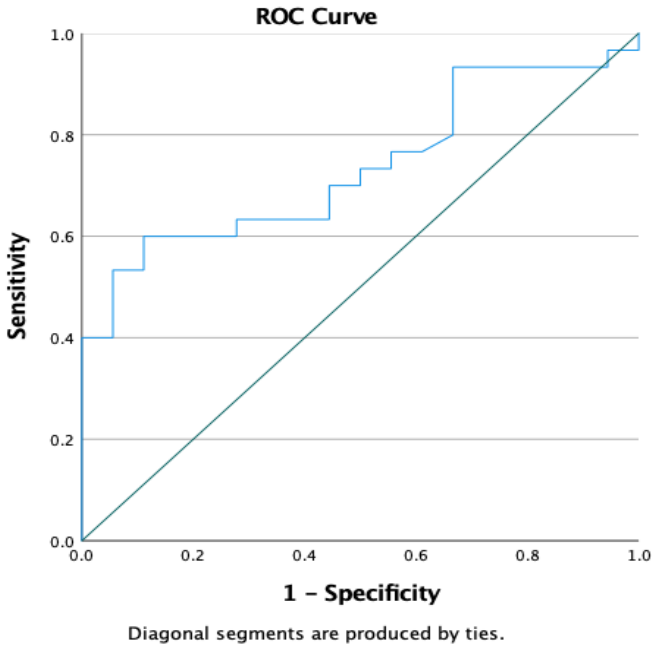


Figure 3.9: ROC curves for Chemerin in arrhythmia patients to analyse the optimal diagnostic points for predicting *of* SVT without risk factor cases compared to other group.

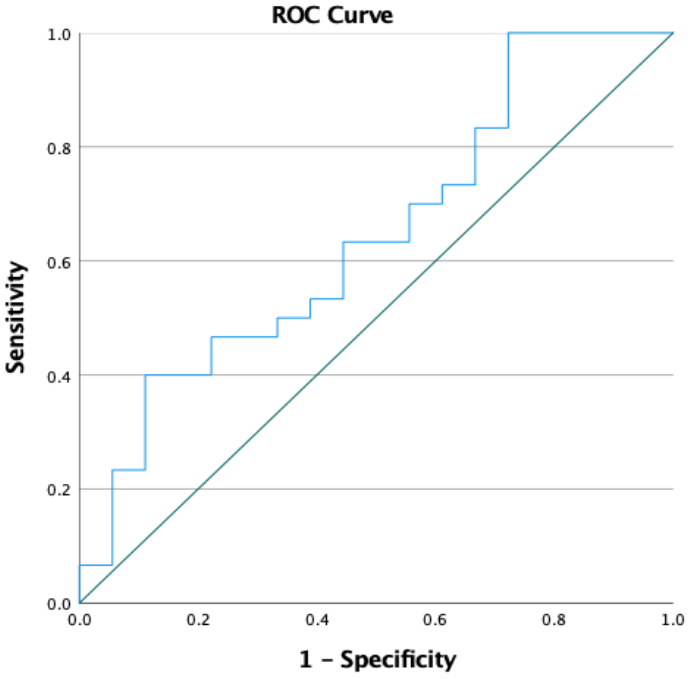


Figure 3.10: ROC curves for Chemerin in arrhythmia patients to analyse the optimal diagnostic points for predicting of *of* SVT without risk factor cases compared to other group.

3.6 ROC curves for Chemerin /Nesfitin ratio in arrhythmia patients to analyse the optimal diagnostic points for predicting of SVT without risk factor etiology of SVT cases compared to SVT with risk factor group

Results of this study confirmed the association of chemerin to SVT arrhythmia cases. To the best of our knowledge, this is an unique study about the analysis of the optimal diagnostic points for predicting arrhythmia (SVT) cases. Previously, Chemerin levels were reported to elevated in metabolic syndrome patients with CAD (132). Further study reported chemerin mRNA expression in epicardial adipose tissue to be associated with coronary atherosclerosis (133). Also, chemerin indicated to be expressed in the affected vascular smooth muscle and foam cells of atherosclerotic lesions and fat enveloping arteries. Also, chemerin during their activation or circulating was activated immune cells or thrombocytes, excreting (134).

During the last years, an increasing number of clinical studies have investigated the relation of circulating chemerin levels with heart disease. In line with our observations, a recent study that analyzed data from uncertain etiology of SVT cases compared to certain group, and healthy patients, roc analysis has indicated an association of serum chemerin with uncertain etiology of SVT cases. to date, the relation between circulating chemerin, and their ratio with Nesfatin has not been examined by any clinical studies.

Investigations have mainly reported on the strong correlation between inflammatory factors and disease development (135). Moreover, inflammation factor could be utilized as a predictive marker of development or recurrence of AF. Therefore, inflammation plays a key role in AF development and progression. studies have demonstrated the important role of nesfatin-1 in inflammation inhibition. Nesfatin-1 injection contributed to the reduced expression of nuclear factor kappa-B, and reduced levels of TNF-α, IL-1β, and IL-6. These results

indicate the anti-inflammatory role of nesfatin-1. However, the exact mechanism of nesfatin-1 in AF development should be investigation in further study (136).

This study was also focused on the potential biomarkers associated with an increased pro-inflammatory status and decrease antioxidant activity; pro-inflammatory status could be characterized by an overactivation of the inflammatory cascade which is simply reflected by the Chemerin /Nesfatin ratio. ROC analysis of Chemerin /Nesfatin ratio indicated that these biomarkers might be a reliable result for this purpose. In spit the role of individual markers, it might be a tool of inflammation in SVT cases along with the previous reported markers.

Final conclusion

&

future work

5.1 Conclusion

- Nesfatin-1 and chemerin levels are affected by SVT arrhythmia disease when adjusted for other cofounders. The present results suggest that serum chemerin can be used as an inflammatory marker of SVT arrhythmia patients as it has good sensitivity and specificity.

- Suggests that Chemerin is strongly associated with markers of inflammation. To date, the ambiguous role of Chemerin in inducing 'inflammation' is still debatable with divided opinions. In addition, along with studying additional Chemerin levels in human health and disease, more research is required to understanding their role.
- Binary logistic regression was performed. It was found that chemerin in certain and uncertain etiology SVT patient was independent risk factors. while Nesfitin was independent protective factors for arrhythmia SVT patient.
- The correlation study shows many significant correlations among the measured parameters, serum Nesfitin levels which was negatively related to the *Chemerin levels.*
- Results of the receiver operating curve (ROC) curve and AUC analysis for the the Nesfitin and chemerin besides their ratio as possible diagnostic parameters. only chemerin and chemerin/ Nesfitin ratio were shown a good diagnostic performance for prediction arrhythmia Patients compared to control group.
- Results were indicated that chemerin was demonstrated the most interesting prediction about uncertain etiology of SVT cases

5.2 Future work:

- Since It has been reported that Certain adipokines are strongly involved in the heart contraction. Serum nesfatin-1 & chemerin might be involved in other types of major adverse cardiac events such as abnormal impulse formation and conduction disturbances.

- It might be a good idea to study the role of other adipokines such as serum resistin, visfatin and leptin in SVT cases

- There is a need to highlight the relevance of these adipokines within their complex cellular environment and cardiac channelopathies disease.

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Appendix

Questionnaire

Nam;e :
Age
Gender

BMI

Weight:
Height:

Hr: …….

Smoking : Yes No

DM: Yes No

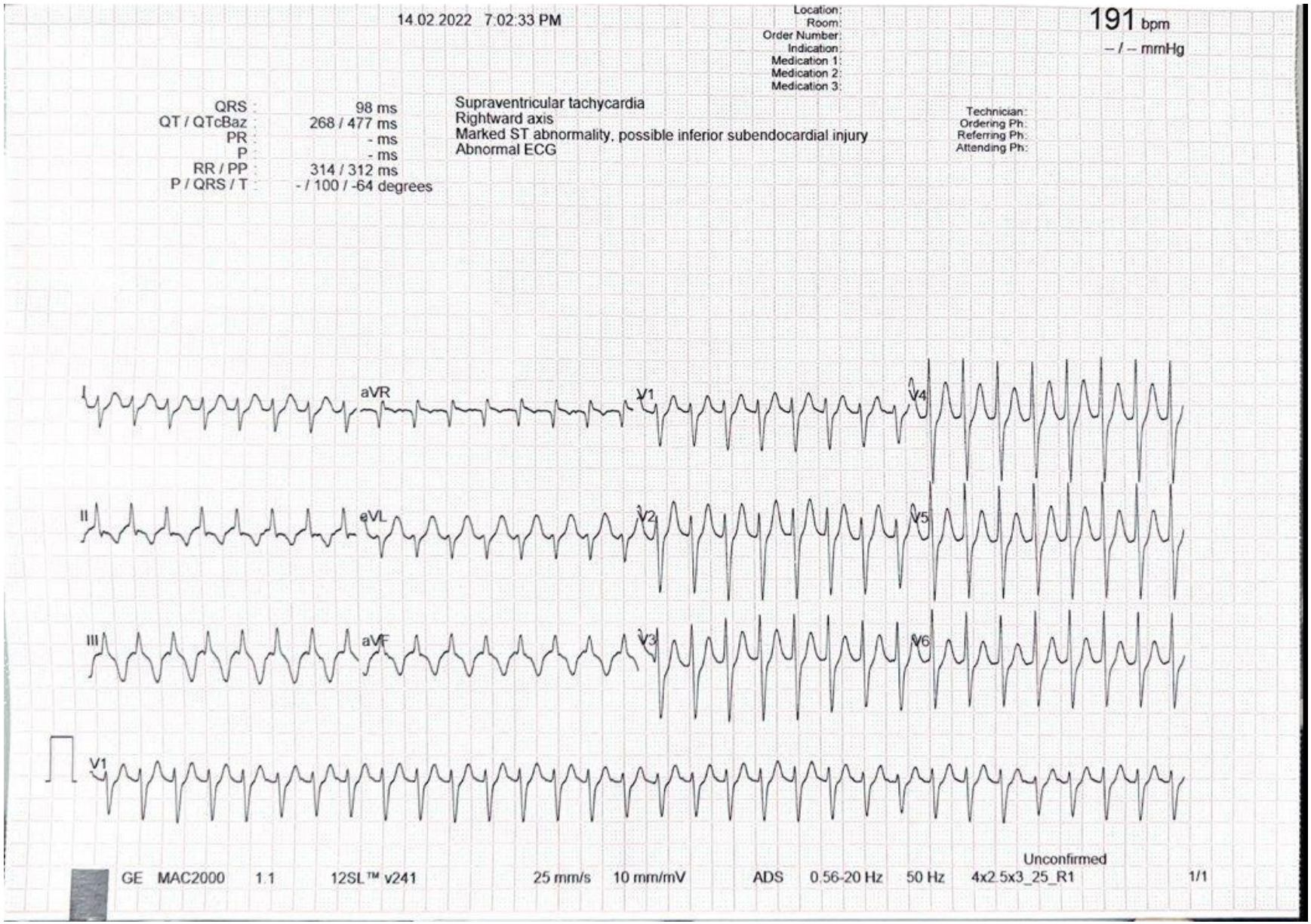
HT: Yes No

endocrine disease: Yes No

hypertension: Yes No

Does the patient have previous illnesses or is it the first time? Yes. No

Does the patient have a family member with this disease? Yes No


14.02.2022 7:02:33 PM
Location:
Room:
Order Number:
Indication:
Medication 1:
Medication 2:
Medication 3:
191 bpm
– / – mmHg
QRS : 98 ms
QT / QTcBaz : 268 / 477 ms
PR : - ms
P : - ms
RR / PP : 314 / 312 ms
P / QRS / T : - / 100 / -64 degrees
Supraventricular tachycardia
Rightward axis
Marked ST abnormality, possible inferior subendocardial injury
Abnormal ECG
Technician:
Ordering Ph:
Referring Ph:
Attending Ph:
I
aVR
V1
V4
II
aVL
V2
V5
III
aVF
V3
V6
V1
Unconfirmed
GE MAC2000 1.1 12SL™ v241 25 mm/s 10 mm/mV ADS 0.56-20 Hz 50 Hz 4x2.5x3_25_R1 1/1

b

الملخص

الخلفية: الأمراض القلبية الوعائية ، غالبًا ما يكون عدم انتظام ضربات القلب وهو احد أنواع مسؤول نسبة كبيرة عن الوفيات المفاجئة ويشير إلى أمراض أخرى عالية الخطورة. عدم انتظام ضربات القلب هو مصطلح جماعي لمجموعة متنوعة من الحالات التي تنطوي على إيقاع القلب بخلاف إيقاع يتم إنتاج الأديبوكينات من الأنسجة الدهنية ، والتي تعتبر أعضاء غدد صماء تشارك في وظيفة القلب والأوعية الدموية التي تسبب تأثيرات فيزيولوجية كهربية مثل تحديد الملامح الأيونية ، والانقباض في الأذين ، والتغيير في شكل إمكانات الفعل Nesfatin-1 .و chemerin هما من الأديبوكين المكتشف حديثًا ، المرتبط بعملية الالتهاب.

تهدف الدراسة الحالية إلى فحص القدرة التشخيصية Nesfatin-1)و (chemerin ونسبتها في حالات عدم انتظام ضربات القلب تسارع فوق البطين.

المواد والطرق: جندت الدراسة 60 مريضًا و 30 الاصحاء. تم تقسيم هؤلاء المرضى إلى مجموعتين فرعيتين من SVT ، محدود السبب لحالات تسارع فوق البطين مع تاريخ واضح لواحد أو أكثر من عوامل الخطر مثل (اضطراب الدهون ، اضطراب الغدة الدرقية ، DM، ارتفاع ضغط الدم الشرياني ... إلخ) ، وغير محدود السبب المؤكدة لحالات SVT بدون أي تاريخ سابق. تم قياس مصل Nesfatin-1 والكيميرين باستخدام تقنية .ELISA تم أيضًا تحديد بعض المعلمات ذات الصلة وربطها بمستوى هذه الأديبوكينات.

النتائج: كان متوسط مستوى المصل nesfatin-1 أعلى بشكل ملحوظ في الأشخاص العاديين منه في كل من مجموعات مسببات SVT المحددة وغير محددةةجة. تم توجيه مخطط تقدير لمستوى مصل Chemerin إلى مرضى SVT غير المؤكد مقارنة بالمجموعات الأخرى.

تم إجراء الانحدار اللوجستي الثنائي. وجد أن الكيميرين في مريض SVT محدد وغير مؤكد (OR: 1.008 و 1.018 ؛ 95٪ (1.018 - 0.998) :CI، (1.028 - 1.008)على التوالي ، كانت عوامل خطر مستقلة. بينما Nesfitin (OR: 0.960 و 0.946 ، 95 ٪ (0.992 - 0.929) :CI، (0.980 - 0.913)كانت عوامل وقائية مستقلة لمريض عدم انتظام ضربات القلب .SVT كانت نتائج منحنى تشغيل المستقبل(ROC) وتحليل AUC لنقاط التشخيص المثلى للتنبؤ المسببات غير المؤكدة لحالات .SVT أشار إلى أن الكيميرين أظهر التنبؤ الأكثر إثارة للاهتمام (الحساسية = 0.60٪ ، النوعية = 0.89٪) عند مستوى = 733.55.(

الاستنتاجات: تتأثر مستويات Nesfatin-1 و chemerin بمرض عدم انتظام ضربات القلب SVT عند تعديلها لمؤسسين آخرين. تشير النتائج الحالية إلى أنه يمكن استخدام كيمياء المصل كعلامة التهابية لمرضى عدم انتظام ضربات القلب SVT نظرًا لأنه يتمتع بحساسية وخصوصية جيدة.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

(التقييم البيوكيميائي لاضطرابات عودة الاستقطاب القلبي لدى مرضى عدم انتظام ضربات القلب)

رسالة ماجستير

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

من قبل

احمد عبد الحميد جواد

بكالوريوس علوم كيمياء 2017

إشراف

أ.م.د. رنا مجيد حميد

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2022

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