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# Evaluation of Serum Ghrelin and Some Biochemical Factors in Type2 Diabetic Iraqi Patients with Cardiovascular Disease

A thesis

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**By**

**Hamsa Ghawamer Ghazy**

B.Sc. Chemistry (2016)/ College of Education for pure Sciences/ Ibn Al-Haitham/ University of Baghdad

**Supervised By**

**Dr. Nawal. M. J. Al-Shammaa**

**Assistant Professor**

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بسم الله الرحمن الرحيم

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I certify that this thesis was prepared under my supervision at the Department of Chemistry, college of Education for Pure Science (Ibn-Al-Haitham) , University of Baghdad as partial requirements for the Degree of Master of Science in Chemistry.

Signature:

Name : Assist. Prof. Dr. Nawal. M. J. Al-Shammaa

Date : 4 / 12 2019

In view of the available recommendation. I forward this thesis for debate by the Examining Committee.

Signature:

Name : Prof. Dr. Mohamad J. Al-Jeboori

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# Certification

We, the examining committee, after reading this thesis " **Evaluation of Serum Ghrelin and Some Biochemical Factors in Type2 Diabetic Iraqi Patients with Cardiovascular Disease** " and examining the student " **Hamsa Ghawamer Ghazy** " in its contents, find that it is qualified for the degree of master of science in Biochemistry with grade of (  ) on (  /  / 2019).

Charime

Signature:

Name: Dr. Taghreed Uloom Mohammed

Professor

Date 4 / 12 / 2019

Memder

Signature: Hind

Name: Dr. Hind Shakir Ahmed

Assistant Professor

Date 4 / 12 / 2019

Memder

Signature: T. AlSaatoon

Name: Dr. Taghreed Abd AL-Hamid Nasser

Assistant Professor

Date 4 / 12 / 2019

Supervisor

Signature:

Name: Dr. Nawal M. Jwad

Assistant Professor

Date 4 / 12 / 2019

Approved by the dean of the college of education (Ibn AL-Haitham)

**Signature** Firas A. Alobaidy

**Name: Assist. Prof. Dr. Firas Abdulhameed Abdullatif**

**Behalf/ The Dean of College of Education**

**For Pure Science/ Ibn al-Haitham**

Data: 8/12/2019

# *Dedication*

***Thanks to Allah most merciful***

***I would like to dedicate my effort to:-***

***My first and last love in this whole world, I can't forget your support and your encouragement to me to believe in myself… My father***

***For your love, patience and sacrifices… I can't express my love to you and gratitude for everything you did for me… My mother***

***The one who helped me to collect the stones from my way to build a successful stair rather than to be hindered by her… My husband***

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# *Abbreviations*

| Brief | Term |
|---|---|
| ADA | American Diabetes Association |
| ADP | Adenosine Diphosphate |
| AGEs | Advanced Glycation End Products |
| AgRP | Agouti-Related Protein |
| AKI | Acute Kidney Injury |
| AMI | Acute Myocardial Infarction |
| AMP | Adenosine Mono Phosphate |
| AMPK | Activated Protein Kinase |
| Ang II | Angiotensin II |
| Anti-cTnI | Antibody Cardiac Troponin I |
| ARC | Arcuate Nucleus |
| Apo B | Apolipoprotein B |
| ATP | Adenosine Tri Phosphate |
| AT1 | Angiotensin II Receptor Type 1 |
| AVP | Arginine Vasopressin |
| BMI | Body Mass Index |
| CA | Coronary Arteries |
| CaMKK | Calcium/Calmodulin-Dependent Protein Kinase Kinase 2 |
| CE | Cholesterol Esterase |
| CHF | Chronic Heart Failure |
| CK-MB | Creatine Kinase-MB |
| CNS | Central Nervous System |
| CO | Cholesterol Oxidase |
| CORS | Cortisol |
| CV | Cardiovascular |
| CVD | Cardiovascular Disease |

| | |
|---|---|
| DAP | Dihydroxy Acetone Phosphate |
| DKD | Diabetic Kidney Diseases |
| DM | Diabetes Mellitus |
| DSBmT | Disulfobutylmetatoluidine |
| ECG | Electrocardiogram |
| EDTA | Ethylenediaminetetraacetic acid |
| FER | Ferritin |
| ELISA | Enzyme Linked Immunosorbent Assay |
| ERS | Endoplasmic Reticulum Stress |
| FPG | Fasting Plasma Glucose |
| GDM | Gestational diabetes mellitus |
| GH | Growth Hormones |
| Ghrelin | Growth Hormone Release Inducing |
| GHSR | Growth Hormone Secretagogue Receptor |
| GHS-R1a | Growth Hormone Secretagogue Receptor Type 1a |
| GK | Glycerol Kinase |
| GLU | Glucose |
| GOAT | Glutamate Oxaloacetate Transaminase |
| GOD | Glucose Oxidase |
| GPO | Glycerol Phosphate Oxidase |
| GPD | Glucose Peroxidase |
| HBA | Hydroxyl Benzoic Acid |
| HF | Heart Failure |
| hsCRP | High Sensitivity C-Reactive Protein |
| HDL-C | High Density Lipoprotein Cholesterol |
| HRP | Horseradish Peroxidase |
| IGT | Impaired Glucose tTolerance |
| IR | Insulin Resistance |
| LDH | Lactate Dehydrogenase |
| LDL-C | Low Density Lipoprotein cholesterol |

| | |
|---|---|
| LDL-s | Low Density Lipoprotein Particles |
| MI | Myocardial Infarction |
| MODY | Maturity-Onset Diabetes of the Young |
| MRI | Magnetic Resonance Imaging |
| mRNA | Messenger Ribonucleic Acid |
| NPY | Neuropeptide Y |
| NTS | Nucleus of TractusSolitarius |
| OD | Optical Density |
| OGTT | Oral Glucose Tolerance Test |
| PKC | Protein Kinase C |
| POMC | Pro-OpioMelanoCortin |
| RAAS | Renin-Angiotensin-Aldosterone System |
| SNA | Sympathetic Nerve Activity |
| SNS | Sympathetic Nervous System |
| T | Testosterone |
| TC | Total Cholesterol |
| TCA | Trichloroacetic acid |
| TD | Testosterone Deficiency |
| T1DM | Type 1 Diabetes Mellitus |
| T2DM | Type 2 Diabetes Mellitus |
| TES | Testosterone II |
| TG | Triglyceride |
| TMB | *Transports Metropolitans de Barcelona* |
| Tn | Troponine |
| VLDL-C | Very Low Density Lipoprotein cholesterol |
| 4-AAP | 4-Aminoantipyrine |
| 4-CP | 4-Chlorophenol |
| 4 PL | 4 Parameter Logistics |

# ABSTRACT

Diabetes mellitus is a disease characterized by disorder in metabolism and abnormally high blood glucose resulting from low levels of insulin with or without irregular resistance to the action of insulin. Diabetes is a prime risk factor for cardiovascular disease. Diabetic complications account for much of the morbidity and mortality in diabetes. Disturbances in lipid profile have been one of the major contributors to cardiovascular disease.

Ghrelin, a peptide hormone is accountable for alteration in energy balance influenced by growth hormone secretion and nutrition. Furthermore, it controls glucose metabolism. Revisions have proposed that ghrelin levels change in diverse functional disorders such as heart diseases and diabetes mellitus.

This study included patients who attended Ibn–Al Nafees Teaching Hospital and Iraqi center of Myocardial Infarction, Baghdad Teaching Hospital from December 2018 to February 2019.

Patients with end-stage myocardial infarction were designated with and without type 2 diabetes. The study contains 100 patients (50 with diabetes and 50 without diabetes), their ages ranges were 40 to 65 years. The patients with myocardial infarction were diagnosed based on from medical reports, laboratory and clinical tests counselor heart disease. Thus, the results of myocardial infarction patients were equated with (50) healthy age coincide (35-65) years. The control group was selected as healthy and not suffering from heart diseases, diabetes, or hypertension.

The present study included numerous parameters e.g. fasting serum glucose, serum lipid profile, serum insulin, ghrelin, ferritin, cortisol, testosterone, urea, creatinine, and have defined their relationship between

serum ghrelin and these factors. All these factors have a major causative role in the pathogenesis of cardiovascular disease in these patients.

The results showed a significant increase in body mass index, fasting serum glucose, total cholesterol, triglyceride, low density lipoprotein cholesterol, serum urea, creatinine, ferritin, and cortisol in myocardial infarction patients with diabetes as paralleled to those without diabetes, while there was a significant decrease in myocardial infarction patients with diabetes as paralleled to those without diabetes.

Myocardial infarction patients (with/without diabetes) had lipid abnormalities, insulin resistance, low levels of serum ghrelin and testosterone, disturbance of urea and creatinine.

The aim of current study was evaluation of serum ghrelin in CVD (with / without) type 2 diabetic patients and, evaluation of ferritin, cortisol, testosterone, insulin levels sera lipid profile and compared them with health individuals.

# Chapter One

## Introduction

## 1.1 Diabetes Mellitus:

Diabetes mellitus (DM) has been known as a heterogeneous metabolic disorder characterized by the presence of hyperglycemia due to impairment of insulin secretion, defective insulin action or both. The chronic hyperglycemia of diabetes is associated with relatively specific long-term microvascular complications affecting the eyes, kidneys and nerves, as well as an increased risk for cardiovascular disease (CVD) [1]. Signs and symptoms of DM are listed in Table (1-1). Often symptoms are not severe or may be absent [2].

**Table (1-1): Signs and symptoms of DM [2]**

| |
|---|
| Polyuria |
| Polydipsia |
| Polyphagia |
| Blurred vision |
| Extreme hunger |
| Impairment of growth and vulnerability to definite infections such as skin infections |
| Unexplained weight loss |
| Ketoacidosis or non-ketotic hyperosmolar syndrome |

In diabetes, glucose in blood cannot move efficiently into cells, so blood glucose levels remain high. This not only starves all the cells that need the glucose for fuel, but also harms certain organs and tissues exposed to high glucose levels [3].

Several pathogenic processes are concerned in the development of DM. These vary from autoimmune destruction of the β-cells of the pancreas with ensuing insulin insufficiency to abnormality that result in

resistance to insulin action. The basis of the abnormalities in carbohydrate, fat, and protein metabolism in DM is incomplete action of insulin on target tissues, which outcome from insufficient insulin secretion and/or diminish tissue response to insulin at one or more data in the multifaceted pathways of hormone action [4].

## 1.1.2. Diabetes Classification:

Diabetes can be categorized into the subsequent common classifications:

- Type 1 diabetes mellitus (T1DM) due to autoimmune β-cell destruction, usually foremost to absolute insulin deficit.

- Type 2 diabetes mellitus (T2DM) due to a progressive loss of β-cell insulin secretion recurrently on the background of insulin resistance (IR).

- Gestational diabetes mellitus (GDM) diagnosed in the $2^{nd}$ or $3^{nd}$ trimester of pregnancy that was not noticeably overt diabetes former to gestation and other particular types [5].

- Specific types of diabetes due to other causes, e.g., monogenic diabetes syndromes (for instance neonatal diabetes and maturity-onset diabetes of the young [MODY]), diseases of the exocrine pancreas (for instance cystic fibrosis and pancreatitis), and drug- or chemical-induced DM [6].

### 1.1.2.1. Type 2 Diabetes Mellitus:

Type 2 DM (T2DM) is the most common type of diabetes which is formerly known as an adult-onset diabetes. This type of diabetes is a disturbance in metabolism and it is influenced by a number of factors; mainly by two distinguished defective ways. Either pancreas decreases

the secretion of insulin, or insulin is normally secreted but resistance develops in its action in body tissue which leads to increased level in blood. Several factors may play role in IR such as genetics, age, fat or excessive glucose consumption [7]. As a result of IR, pancreatic β-cells start producing more and more amount of insulin. The continuous resistance in the action of insulin and increase of its concentration may lead to the decrease in its production and eventually the extracellular hyperglycemia [8]. Patients with this type of DM do not need insulin as a therapeutic to survive, so this type was previously called none insulin dependent diabetes. Most type 2 diabetic patients are obese, and obesity itself causes some degree of IR. Patients who are not obese by traditional weight criteria may have an increased percentage of body fat distributed predominantly in the abdominal region. The high prevalence of diabetes and its associated multiple complications, such as: CVD, blindness, renal failure, lower extremity amputations, and premature death, lead to an enormous overall burden of disease [9].

### 1.1.3 Diagnostic Tests for Diabetes Mellitus:

The recognition of patients with DM or pre-DM by screening allows for earlier intervention, with possible reductions in prospect complication rates, though randomized trials are missing to definitively show benefit. The patient described in the vignette has risk factors like hypertension, obesity and a family history of DM and might be screened [10]. Around 25% of patients with T2DM already have microvascular complications at the time of diagnosis postulating that they have had the disease for more than 5 years at the time of diagnosis [11]. As a consequence there are diverse approaches to diagnose DM among subjects. The American Diabetes Association (ADA) recommendations for diagnosis of DM focal

point on fasting plasma glucose (FPG), while WHO focuses on the oral glucose tolerance test (OGTT) [12].

### 1.1.4 Complications of Diabetes Mellitus:

Elevation of blood glucose level for a long time causes serious complications. Long term or irreversible complication due to DM is abnormality in eyes with complete loss of vision, renal failure and nerve damage of peripheral region with damage at the end of organ. Abnormality in the autonomic nervous system causes abnormal functioning of gastrointestinal tract, urinary tract and also cardiovascular (CV) system [13]. Diabetic patient is also at increased risk of atherosclerotic CV, peripheral arterial and cerebrovascular disease. Hypertension and abnormalities of lipoprotein metabolism are often found in people with DM [14].

#### 1.1.4.1 Cardiovascular Diseases:

Cardiovascular diseases are the class of diseases that involve the heart or blood vessels. While the term technically refers to any disease that affects the CV system, it is usually used to refer to those related to atherosclerosis. These conditions usually have similar causes, mechanisms, and treatments [15]. By the time that heart problems are detected, the underlying cause atherosclerosis is usually quite advanced, having progressed for decades. Atherosclerosis is a slow progressive disease that begins with the deposition of lipids in the walls of large blood vessels, particularly the coronary arteries (CA) [16]. Although a very large plaque can occlude the lumen of the artery, blood flow is usually not completely blocked unless the plaque ruptures. This triggers formation of a blood clot that can prevent circulation to the heart, causing

myocardial infarction (MI) or acute myocardial infarction (AMI), commonly known as a heart attack [17]. This is most frequently due to blocking of a coronary artery after the rupture of a vulnerable atherosclerotic plaque, which is an unstable collection of lipids (cholesterol and fatty acids) and white blood cells especially macrophages in the wall of an artery. The resultant ischemia and ensuing oxygen shortage, if left untreated for a sufficient period of time, can cause damage or death of heart muscle tissue. Symptoms of AMI include sudden chest pain typically radiating to the left arm or left side of the neck, shortness of breath, palpitations, sweating, nausea, vomiting, and anxiety [18].

Among the diagnostic tests available to detect heart muscle damage are an electrocardiogram (ECG), echocardiography, cardiac magnetic resonance imaging (MRI) and various blood tests. The diagnosis of AMI depends largely on cardiac markers in blood such as creatine kinase-MB isoenzyme (CK-MB), creatine kinase-total, troponins and myoglobin [19].

### 1.1.4.1.2 Diabetes Mellitus and Cardiovascular Disease:

Diabetes is a main risk factor for the development of atherosclerosis. In addition to improved risk of MI, stroke, and peripheral vascular disease, diabetics undergo from a principally destructive form of atherosclerosis with larger in-hospital mortality subsequent MI and an elevated occurrence of heart failure, if they survive [20]. While diabetics frequently have other associated risk factors for atherosclerosis e.g., obesity, hypertension, hypercholesterolemia, the further risk conferred by DM and the principally destructive vascular and MI that affects diabetics

recommend that diabetes-related atherosclerosis includes distinctive pathogenic mechanisms [21]. In fact, CVD is a main reason of morbidity and mortality in type 2 diabetic patients. Individuals who have several blockages of their CA, if they as well have DM which may promote from bypass surgery [22].

## 1.2 Hormones Regulates Glucose Metabolism:

### 1.2.1 Insulin:

Insulin is a hormone promotes anabolism that balances subjects caloric needs and intake with expenditure. Human insulin is a peptide hormone composed of 51 amino acids and has a molecular weight of 5808 Da. Circulating and biologically active for insulin is monomeric [23].

The pathologic at which target cells fail to respond to ordinary levels of circulating insulin is called IR. Clinically, IR is defined as the inability of endogenous or exogenous insulin to lower blood glucose and to maintain metabolic homeostasis [24].

Insulin capability to stimulate the disposal of glucose differs at least six fold in healthy individuals, and about one-third of the population that is most resistant to this action of insulin is at greatly risk to develop a number of complications. Type 2 DM was the first clinical syndrome that identified as being related to IR because it occurs when pancreas individuals are unable to secrete enough insulin to compensate for the defect in insulin action [25].

The IR is closely associated with several factors including hyperglycemia, abnormal lipid profiles and alterations in inflammatory

mediators which considered as risk factors for CVD in type 2 diabetic patients [26].

Fat accumulation, usually due to overfeeding, overfills the present subcutaneous fat cells, and leads to fat accumulation in the abdomen, the visceral fat, muscles, and liver. The adipocytes in the visceral fat start to produce many adipokines, which alter different metabolic processes: serum lipids modify, elevated of blood pressure, purine and estrogen levels, reduced testosterone levels, the thyroid gland may start to dysfunction, and the production of insulin increases to 20 times the normal level (hyperinsulinemia) [27]. After a longer period of time, the pancreas fails in meeting insulin requirements after meals, foremost to impaired glucose tolerance (IGT), and lastly to T2DM [28].

### 1.2.2 Growth Hormone Release Inducing:

Growth Hormone Release Inducing (Ghrelin) was identified in 1999 in a study which was designed to search for an endogenous ligand for an orphan receptor, the type 1a growth hormone secretagogue receptor (GHS-R1a) [29]. Ghrelin is a hormone formed principally by focused cells of stomach called P/D1 cells present in the lining of stomach and is secreted in small amount from epsilon cells of pancreas. In Pituitary gland, hypothalamus, kidney, placenta and brain also have smaller amount of ghrelin. Ghrelin elevates appetite by acting on the hypothalamus which is a fraction of the brain control appetite and promotes fat storage [30]. A further significant function of ghrelin is that it promotes the growth hormones (GH) secretion from the anterior pituitary gland by binding to its specific receptors GHSR present in the anterior pituitary and these receptors have also been originate in adipose tissue, heart and hypothalamus [31]. Ghrelin is appetite-stimulating and

GH releasing peptide and it has been given the nickname, the -hunger hormone [32].

Apart from its effect on GH, it has various important biological actions like regulation of CV. Ghrelin has prokinetic effects in the gastrointestinal tract and is thought to play an anti-inflammatory role [33]. In addition, there is convincing evidence that ghrelin plays a role in insulin release and glucose homeostasis. There remains much debate on the effects of ghrelin on insulin signaling, and on its role in DM, and these topics are modulating cell proliferation and survival, energy balance and metabolism and plays a key role in the control of insulin release [34].

Microinjection of ghrelin into this nucleus considerably reduced the mean arterial pressure and heart rate. This injection also inhibited sympathetic activity. Thus, the regulation of insulin secretion by ghrelin is strongly associated to the blood glucose level. Additionally, the novel octanoylated structure of ghrelin documented as a new discovery in biochemistry. The recently recognized enzyme; ghrelin-O-acyl transferase (GOAT) that catalyzes the acyl-modification of ghrelin, and provides the secretory machinery of the ghrelin and may herald novel development in appreciative of fatty acid metabolism [35].

### 1.2.2.1 Chemical Structure of Ghrelin Hormone:

Ghrelin is a 28-amino acid peptide hormone cleaved from the precursor molecule preproghrelin [36]. It is produced by and released from the stomach in at least two forms: acylated ghrelin and des-acyl ghrelin [37]. Acylation is vital for ghrelin bioactivity and arises through the addition of an octanoyl group to the serine at the third N-terminal position in a reaction catalyzed by GOAT [38]. Acylated ghrelin makes

up approximately 20% of the total circulating ghrelin, with the remainder consisting of des-acyl ghrelin also known as unacylated ghrelin [39]. Ghrelin is most highly expressed in the stomach [40], which is the source of most circulating ghrelin, but lower levels can also be detected in the pancreas, intestine and hypothalamus. Acylated ghrelin binds to and activates GHS-R with a half maximal effective concentration in the low nanomolar range [41]. Summary of non orexigenic of ghrelin and its signaling is illustrated in Figure (1-1).

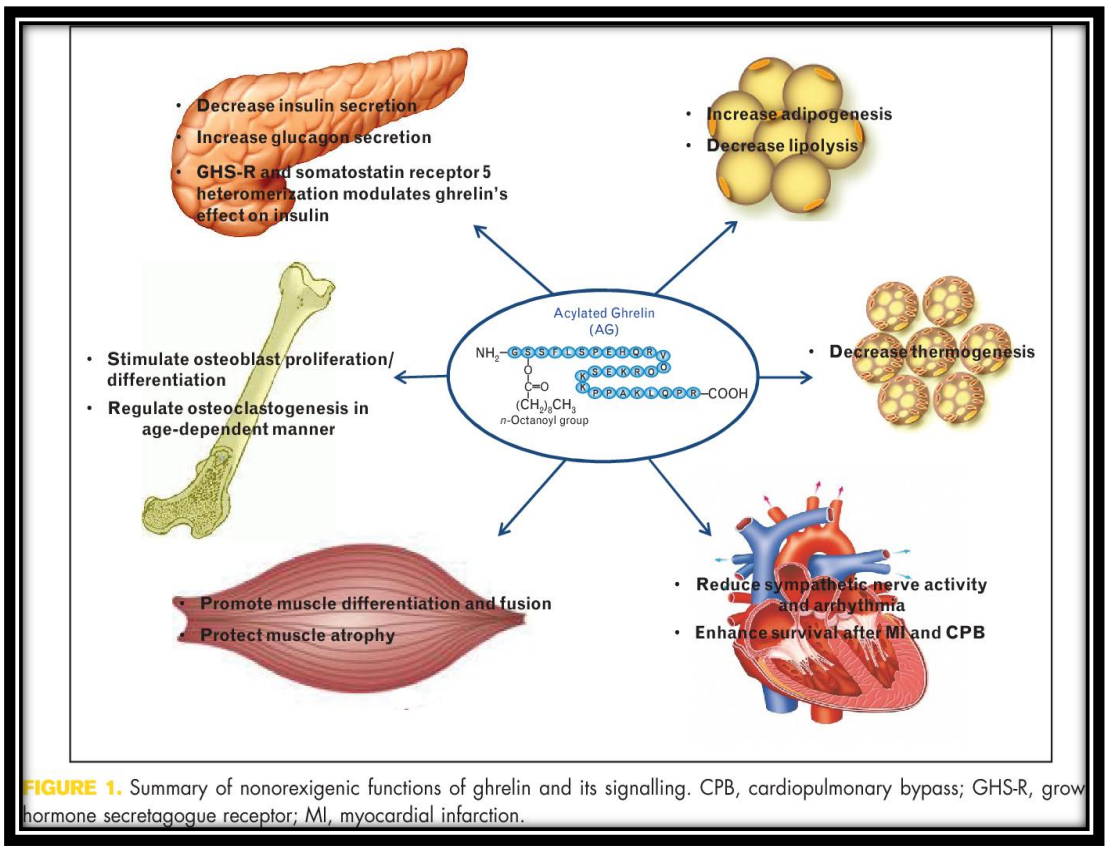


**Figure (1-1): Summary of nonorexigenic functions of ghrelin and its signaling** [41]

Des-acyl ghrelin also binds to GHS-R, but with an efficacy several orders of magnitude lower than acylated ghrelin. The GHS-R is most highly expressed in the arcuate nucleus of the hypothalamus and in the anterior pituitary. It has been suggested that des-acyl ghrelin can exert

some biological effects through an unidentified receptor or via a non-receptor mediated mechanism [42].

### 1.2.2.2 Synthesis and Release of Ghrelin hormone

Approximately 60-70 % of circulating ghrelin is secreted by the stomach, with most of the remainder originating in the small intestine. Low-level ghrelin expression also occurs in several tissues outside the gut, including hypothalamus (arcuate nucleus and paraventricular nucleus), pituitary, lung, adrenal cortex, kidney, bone, testis, placenta and pancreatic islet cells [43].

The human preproghrelin gene is located on chromosome 3p25-26 and consists of five exons with four introns. Spliced ghrelin messenger ribonucleic acid (mRNA) is translated to a 117-amino acid preproghrelin precursor, which is consequently cleaved to produce ghrelin. Additionally, obestatin, a 23-amino acid peptide is a supposed proteolytic fragment of the preproghrelin precursor purified from rat stomach extracts. In contrary to the appetite stimulating effects of ghrelin, treatment of rats with obestatin suppressed food intake, suppressed jejunal contraction and reduced bodyweight gain [44]. Nevertheless the appetite inhibiting influence of obestatin unsuccessful to be established in [45].

The amino acid sequences of mammalian ghrelin are well preserved, principally the 10 amino acids in their $NH_2$ termini, which are identical. This structural conservation and the universal constraint for acyl-modification of the third residue designate that this $NH_2$-terminal region is of central significance to the activity of the peptide. Rat and human

ghrelin vary in only two amino acid residues [46]. Structure of ghrelin and des-acyl ghrelin is shown in Figure (1-2).

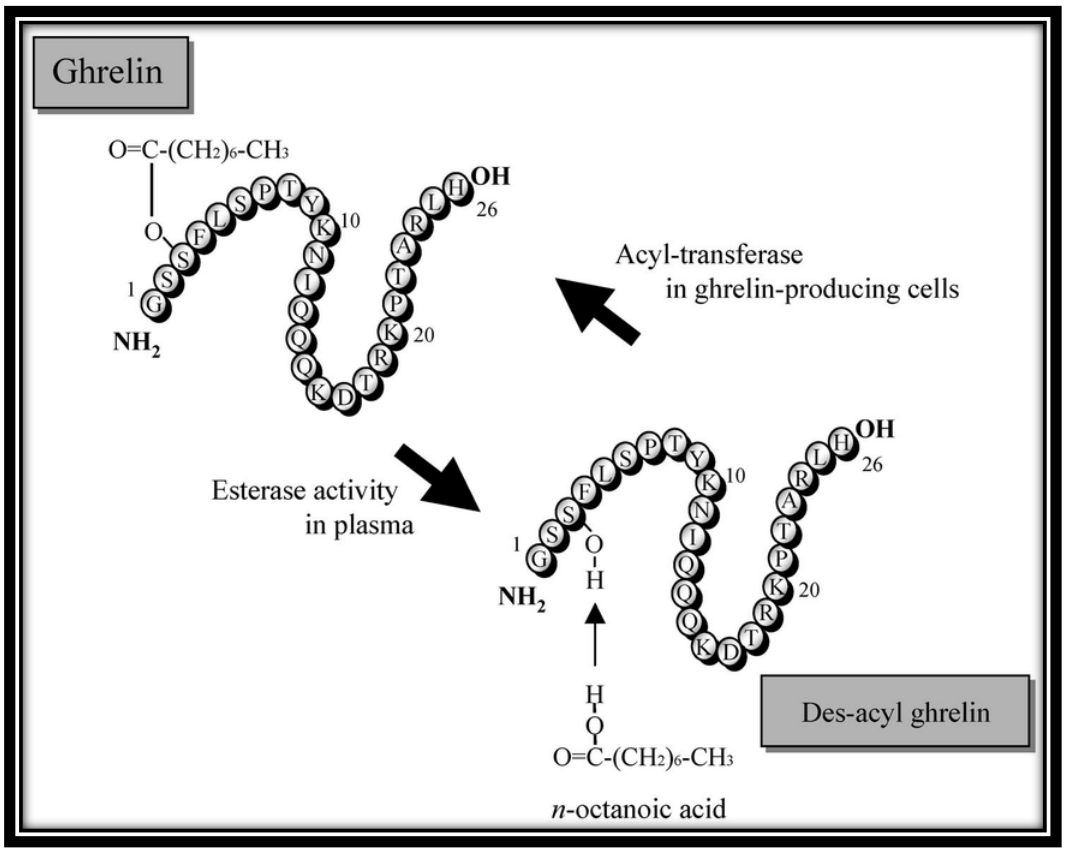


**Figure (1-2): Structure of ghrelin and des-acyl ghrelin[46]**

## 1.2.2.3 Mechanism Action of Ghrelin Hormone:

Ghrelin containing neurons are present in the arcuate nucleus (ARC) of the hypothalamus, a region implicated in appetite regulation [47]. In fact, intracerebroventricular injection of ghrelin increases cumulative food intake and reduced energy expenditure, ensuing in body weight gain [48]. This orexigenic influence of hypothalamic ghrelin is regulated through a neuronal network relating food intake. To motivate the liberate of the orexigenic peptides, ghrelin-containing neurons send efferent fibers onto neuropeptide Y (NPY) and agouti-associated protein (AgRP)-

expressing neurons. Alternatively, to suppress the release of the anorexigenic peptide, ghrelin-containing neurons send efferent fibers onto pro-opiomelanocortin (POMC) neurons [49].

The ARC is as well a target of leptin, an appetite-suppressing hormone formed in adipose tissues. Leptin directly suppressed appetite-stimulating influences of NPY and AgRP, while hypothalamic ghrelin augments NPY gene expression and blocked leptin-induced feeding lessening. Thus, ghrelin and leptin have a competitive relations in feeding regulation [50].

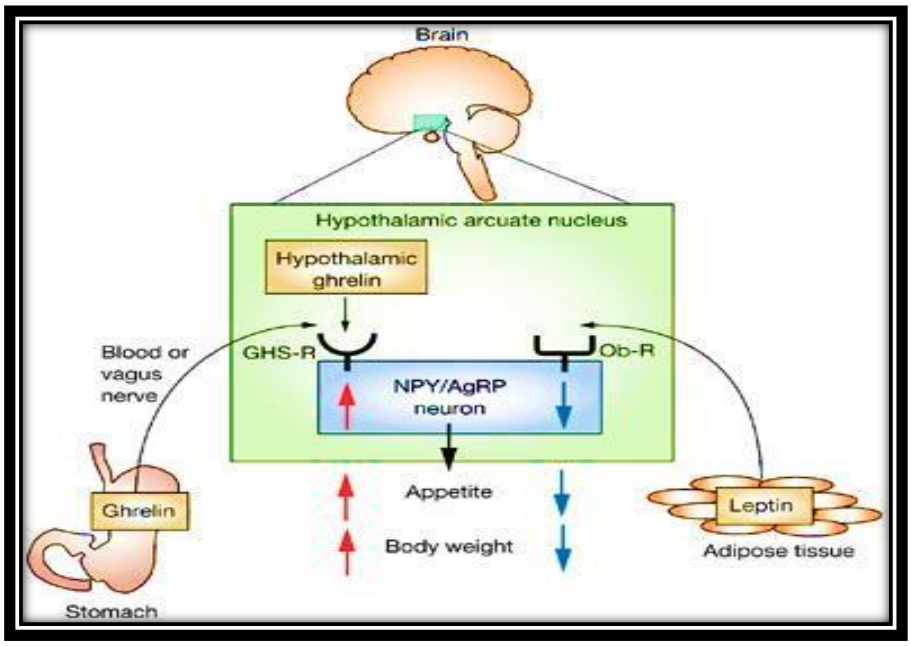


**Figure (1-3): Mechanism action of ghrelin hormone [51]**

## 1.2.2.4 Ghrelin and Cardiovascular Diseases:

Clinical studies have reported that ghrelin confers a variety of potentially beneficial cardiovascular effects [52], which includes reduction of mean arterial blood pressure, increase in myocardial contractility, protection of endothelial cells, and improvement of energy metabolism of myocardial cells [53].

Exogenous administration of ghrelin also results in improvement in coronary flow, heart rate, dilatation of peripheral blood vessels, constriction of coronary arteries, and improvement in ventricular and endothelial function. It has been reported that exogenous administration of ghrelin decreases muscle wasting, improves exercise capacity, inhibits cardiomyocyte apoptosis, inhibits sympathetic nerve activity, and protects from heart failure (HF) induced by MI [54]. Importantly, administration of ghrelin has been demonstrated to improve the cardiac function and prognosis in patients suffering from end-stage chronic heart failure (CHF). In vitro, ghrelin decreases inotropism and lusitropism. Ghrelin reverses cardiac cachexia by promoting a positive energy balance and also by enhancing direct cardioprotective effects of ghrelin [55].

These cardioprotective effects are independent of growth hormone release and likely involve binding to cardiovascular receptors. There is a widespread distribution of ghrelin and its receptors GHS-R1a in the cardiovascular tissues, which provides a definitive evidence of its cardiac actions. The protective effects of ghrelin on heart are mediated through direct effects on the heart and blood vessel and through its growth-hormone-releasing effect [56].

In normal individuals, acute increases in ghrelin do not alter cardiac metabolism, whereas in patients with HF. They enhance oxidation of free fatty acids and reduce the oxidation of glucose, thus partly correcting its metabolic alterations. This interesting mechanism of action of ghrelin may contribute to the cardioprotective effects of ghrelin in HF. Ghrelin mediates cardioprotective effects by modulating cardiac autonomic nervous activity. However the precise mechanisms by which ghrelin regulates sympathetic activity are still unclear and needs further

investigation. Peripheral ghrelin may act on GHS-R1a at the cardiac vagal nerve ending, which goes to the nucleus of tractussolitarius (NTS) and inhibit the renal sympathetic nerve activity (SNA) [57]. Ghrelin can also act directly on the central nervous system (CNS) and alter the sensitivity of CNS to other hormones participating in regulation of sympathetic activity. Administration of ghrelin brings down the plasma levels of epinephrine and dopamine and shifts the balance of autonomic nervous activity toward parasympathetic nervous activity [58]. Ghrelin increases the size of cardiomyocytes, prolongs their survival, and protects the cardiomyocytes against apoptosis and myocardial injury induced by endoplasmic reticulum stress (ERS) through a GHS-R1a, calmodulin-dependent protein kinase kinase (CaMKK), and adenosine monophosphate (AMP)-activated protein kinase (AMPK) pathway [59].

Administration of ghrelin lowers the release of lactate dehydrogenase (LDH) and myoglobin by the cardiomyocytes, indicating protection against cardiomyocyte injury. Moreover, ghrelin may have other cardiovascular beneficial effects in the form of prevention of atherosclerosis as well as protection from ischemia and reperfusion injury [60].

Ghrelin inhibits cardiomyocyte apoptosis both in vivo and in vitro. Ghrelin suppresses the Ang II induced cardiomyocyte apoptosis in patients with HF. Ghrelin also inhibits the angiotensin-II receptor type1 (AT1) receptor up-regulation induced by Ang II, thereby playing a role in preventing HF. Elevated levels of ghrelin in patients with HF can be a protective compensatory mechanism for reduced body weight in order to enhance appetite and weight gain in cachexia patients with HF [61].

## 1.3 The Association of Serum Ferritin and Cardiovascular Diseases:

Ferritin is an iron-containing protein and its assessment in serum can consistently reveal the human iron storage homeostasis which is essential in fundamental metabolic processes in living organisms [62]. Extremely increased ferritin levels (hyper-ferritinemia) may designate iron overload, which is toxic for numerous organs e.g. myocardium, liver etc. and has been related with higher morbidity and mortality [63]. On the other hand, decreased ferritin levels (hypo-ferritinemia) reveals iron depletion, which is also correlated with higher morbidity and mortality [64].

Ferritin, beyond its function as an iron-storage marker, is a multi-functional protein with probable roles not only in iron transfer, but in proliferation, angiogenesis, and immune suppression [65]. It is also an acute-phase protein, whose synthesis is driven by cytokines and its levels elevate in inflammatory situations [66].

There are inconsistent consequences highlighting the pluripotency of ferritin as a biomarker in a diversity of illnesses. Preceding study have found a positive correlation between high serum ferritin concentrations and the progress of chronic diseases, such as CVD, cancer, and their adverse progression [67].

## 1.4 Serum Cortisol as a Cardiovascular Risk factor:

Despite lifestyle improvements and the successful targeting of CVD risk factors, as hypercholesterolemia and hypertension, CVD is a leading cause of death in high income countries and increasingly so in low and middle income countries [68].

The identification of new causal risk factors, such as elevated morning plasma cortisol, has the potential to improve CVD risk prediction and the development of new treatments to reduce CVD deaths. Cortisol is a glucocorticoid produced by the adrenal glands, which is responsible for CV and metabolic adaptations during stress. Plasma cortisol has a circadian rhythm with a peak on waking and then declining throughout the day [69].

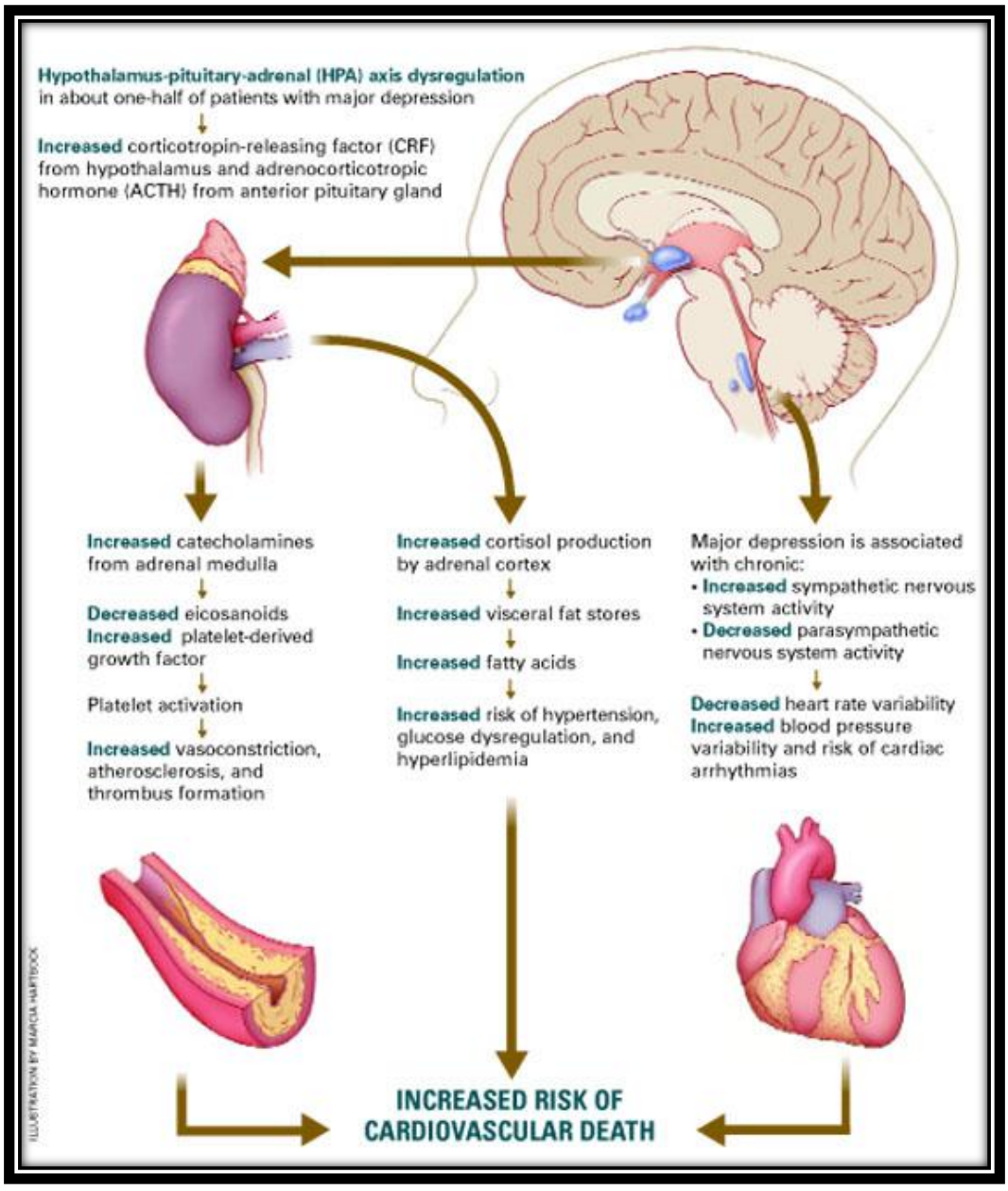


**Figure (1-4): Serum cortisol and risk of CVD [70]**

Inappropriately sustained cortisol production in patients with tumors causing Cushing's syndrome results in abdominal obesity, hyperglycemia, hypertension, and dyslipidemia and is associated with a four-fold increase in mortality rate, predominantly due to accelerated atherosclerosis and increased CVD. A similar higher rate of CVD has been documented in patients prescribed exogenous glucocorticoid therapy at supra-physiological doses [71].

## 1.5 Serum Testosterone and Cardiovascular Diseases:

Testosterone deficiency (TD) is a well-known and substantial medical situation [72].It has been celebrated as a clinical and biochemical syndrome, related with older age and comorbidities. It is categorized by an insufficiency in serum androgen levels, with or without diminished genomic sensitivity to androgens [73]. The latter correlates to the functionality of androgen receptors [74].

Biochemical TD must be related with relevant signs and symptoms for a diagnosis to be made. The hormone has significant physiological functions and deficient can unfavorably affect the fat, bone, muscle, brain, peripheral nerves, the CV system and particularly the male genital and reproductive systems. Testosterone is essential for the regulation of carbohydrate, lipids, and proteins metabolism, and definitely disturbs glucose control, liver fat, cardiac biomarkers, muscle growth and adipogenesis [75].

# *Aim of the Study*

The current study achieve to:

1. Evaluation of serum ghrelin concentrations in type 2 diabetic patients and CVD and compare them with healthy individuals.

2. Assessment of serum glucose, insulin levels, and HOMA-IR in type 2 diabetic patients and CVD and compare them with healthy individuals.

3. Estimation of serum lipid profile, urea, and creatinine in type 2 diabetic patients and CVD and compare them with healthy individuals.

4. Evaluation serum ferritin, cortisol, and testosterone in type 2 diabetic patients and CVD and compare them with healthy individuals.

5. Study the correlation between serum ghrelin levels and other limitations.

# Chapter Two

## Subjects

## &

## Methods

## *2.1 Materials:*

### *2.1.1 Chemicals:*

The common laboratory chemicals used in this study with their suppliers and companies are listed in Table (2-1).

**Table (2-1): The chemicals utilized and their companies**

| Chemicals | Company |
|---|---|
| Ghrelin kit | Elabscience, USA |
| Urea kit | Randox, Crumlin |
| Creatinine kit | Randox, Crumlin |
| Glucose kit | Randox, Crumlin |
| Insulin kit | Abbott, Germany |
| Total Cholesterol kit | Abbott, Germany |
| Triacylglycerol kit | Abbott, Germany |
| High Density Lipoprotein Cholesterol kit | Abott, Germany |
| Cortisol kit | Biomeriux, France |
| Ferritin kit | Biomeriux, France |
| Testosterone kit | Biomeriux, France |

### 2.1.2 Instruments:

Instruments with their companies and countries are listed in Table (2-2).

**Table (2-2): Instruments utilized with their companies and countries**

| Instruments | Company |
|---|---|
| Centrifuge | Hettich, Germany |
| Spectrophotometer | Cecil, English |
| Cobasc | Roche, Germany |
| Minividas | Biomerix, France |
| Abbott | Abbott, Germany |
| Elisa | DRG, Itali |
| Elisa | Human reads, Germany |
| Incubator | Boekel |
| Automatic Pipette | Dragon, China |

## 2.2 Patients Selection and Blood Sampling:

Current study incorporated 100 patients with end-stage MI (50 with DM and 50 without DM), who attended Ibn–Al Nafees Teaching Hospital and Iraqi Center for Myocardial Infarction/ Medical City Hospital- Baghdad during the period from December 2018 until February 2019.Their ages ranged from 40 to 65 years. The MI patients were diagnosed based on their medical reports, laboratory, and clinical tests for heart disease. Also, the results of MI patients were paralleled with 50 healthy individuals age coincide 35-65 years as control group.

Five milliliters of blood samples were taken from each patient in the morning after injury MI, 3days maximum prior to cardiac catheterization. Patients' blood samples were obtained from the needle puncture site for the patients' vein. Serum that obtained was stored at -20℃ until the time of examination. Venous blood samples were also taken from the control group using disposable syringe and centrifuged to obtain serum.

Patients with neuropathy, retinopathy, thyroid dysfunction, and liver diseases were excluded from the study.

## 2.3 Anthropometric Measurements:

### 2.3.1 Measurement of Body Mass Index (BMI):

Body mass index is a simple index of weight-for-height that is ordinarily used to distinguish insufficient weight, normal, overweight and obesity. It is calculated by dividing the weight in kilograms on the height square in meters ($kg/m^2$) presenting to the following equation:-

$$BMI = Weight\ (Kg)\ /(Height)^2\ (m^2)$$

The normal range of BMI was reported between 18 to 24.9 $kg/m^2$, a BMI lower than 18.5 $kg/m^2$suggest that the person is underweight, persons with BMI between (25 to 29.9) $kg/\ m^2$were categorized as overweight and those having 30 $kg/m^2$or more were categorized as obese [76].

## 2.4 Analytical methods and procedure:

### 2.4.1 Determination of Serum Glucose:

**Principle:**

$$\text{Glucose} + H_2O \xrightarrow{GOD} \text{Gluconic acid} + H_2O_2$$

$$2H_2O_2 + \text{4-aminoophenazone} + \text{phenol} \xrightarrow{GPD} \text{Quinoneeimine} + 4H_2O$$

**Procedure:**

The fresh $H_2O$, a new gain calibration was used and completed in cuvette mode. selected GLU in the run test screened and carried out a water blank [77].

Pipette into cuvette:

| | Standard SI or Sample | Reagent Blank S0 |
|---|---|---|
| Sample/Standard | 5µL | 0 µL |
| Reagent RI | 500 µL | 500 µL |

Mix incubate for 10 min at 37℃. Wave length: 500 nm.

## Calculation:

Glucose concentration (mmol/l)=$\frac{Asample}{Astandard}$ × Standard Conc.

**Normal Values:** 4.2-6.4 mmol/l

## 2.4.2 Determination of Serum Insulin:

**Principle:**

The DRG® Insulin ELISA Kit is an enzyme linked immunosorbent assay (ELISA) based on the principle of sandwiches. The microtiter wells were drilled with a monoclonal antibody directed toward a unique antigen site on the insulin molecule. An aliquot of the patient's sample containing the internal insulin was incubated in the well coated with the conjugated enzyme, a biotin-linked insulin antibody, after incubation, conjugate is washed . During the second incubation step, the streptavidin peroxidase enzyme complex binds to the anti-biotin anti-insulin antibody. The amount of complex binding HRP is proportional to the insulin level in the sample. After the addition of the substrate solution, the advanced intensity of the color is proportionate to the level of insulin in the patient sample [78].

**Procedure:**

All standards, samples, and controls were operated simultaneously in order for all test conditions to be the same.

1. The required number of microtiter wells were secured in the holders.

2. Then 25 µl of each standard, controls and samples were dispensed in the wells using disposable pipettes tip.

3.Moreover, 25 µl Enzyme Conjugate were dispensed into each well.

4. It is important to have a complete mixing in this step for 10 seconds.

5. Incubation for 30 minutes at room temperature without covering the plate.

6. The contents of the wells were quickly shake out and rinsed the wells 3 times with diluted wash solution (400 µl per well). the wells were sharply struckon absorbent paper to remove residual droplets.

7.A 50 µl of enzyme complex were added to each well.

8. Incubation for 30 minutes at temperature room.

9. the contents of the wells were quickly shake out and rinsed the wells 3 times with diluted Wash Solution (400 µl per well). Struck the wells sharply on absorbent paper to remove residual droplets.

10. Addition of 50 µl from substrate solution to each well.

11. Incubation for 15 minutes on temperature room.

12. The enzymatic reaction were stopped by adding 50 µl of stop solution to each well, and was read OD at 450 ± 10 nm with a microtiter plate reader within 10 minutes after adding the stop solution

**Calculation:**

1. The average absorbance values were calculated or each set of criteria, controls and patient samples.

2. A standard curve was constructed by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical(Y) axis and concentration on the horizontal (X) axis.

3.The mean absorbance value was used for each sample determined the corresponding concentration from the standard curve.

4. The concentration of the samples could be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted. For the calculation of the concentrations this dilution factor has to be taken into account.

**Normal Values:** 2 to 25 µIU/ml.

## 2.4.3 Determination of Serum Ghrelin:

**Principle:**

ELISA kit uses Competitive-ELISA principle. The micro ELISA plate provided in this kit was pre-coated with Human GHRL. During the reaction, human GHRL in sample or standard competes with fixed amount of human GHRL on solid phase supporter for sites on the biotinylated detection Ab specific to Human GHRL. Excess conjugate and unbound sample or standard were washed from plate, and avidin conjugated to Horseradish Peroxidase (HRP) are added to each microplate well and incubated. Then a TMB substrate solution was added to each well. The enzyme-substrate reaction was terminated by the addition of stop solution and the color change was measured

spectrophotometrically at a wave length of 450 nm ± 2 nm. The concentration of human GHRL in the samples was determined by comparing the OD of samples to the standard curve [79].

**Reagent Preparation:**

**1.** All reagents were brought to room temperature 18-25℃ before use. We follow the microplate reader manual for set-up and preheat it for 15 min before OD measurement.

**2.** A 30 mL of concentrated wash buffer was diluted with 720 mL of deionized or distilled water to prepare 750 mL of wash buffer.

**3.**The standard centrifuge at 10,000×g for 1 min. Then, 1.0 mL of reference standard and sample diluents were added, and let it stand for 10 min and invert it gently several times. This reconstitution produces a working solution of 10 ng/mL. Then the serial dilutions as needed. The recommended dilution gradient was as follows: 10, 5, 2.5, 1.25, 0.63, 0.31, 0.16, 0 ng/mL and dilution method was taken 7 EP tubes, 500uL of reference standard and sample diluent were added to each tube. A 500uL of the 10 ng/mL stock solution was pipetted to the first tube and mixed up to produce a 5 ng/mL working solution. A 500uL of the solution was pipetted from the former tube into the latter one according to these steps.

**4.**The required amount 50 μL/well was calculated before the experiment. The stock tube was centrifuged before use, diluted the 100× concentrated biotinylated detection Ab to 1×working solution with biotinylated detection Ab diluent.

**5.**The required amount was calculated before the experiment 100 μL/well. In preparation, slightly more than calculated should be prepared.

Dilution of 100× concentrated HRP conjugate to 1× working solution with concentrated HRP conjugate diluent.

**Assay procedure:**

**1.**The standard working solution was added to the first two columns: Each concentration of the solution was added in duplicate, to one well each, side by side (50 µL for each well). The samples were added to the other wells (50 µL for each well). Immediately, 50µL of biotinylated detection Ab working solution was added to each well, and covered the plate with the sealer provided in the kit. The mixture was incubated for 45 min at 37℃.

**2.**The solution from each well was decanted, 350 µL of wash buffer was added to each well. The solution was from each well soaked for 1~2 min and aspirate dafter put it dry against clean absorbent paper. This wash step was repeated 3 times.

**3.**A 100 µL of HRP conjugate working solution was added to each well. Overlay with the plate sealer and incubated for 30 minutes at 37℃ .

**4.**The solution from each well was aspirated, the wash practicability was iterated for five times as fulfill in step 2.

**5.**Then 90µL of substrate reagent was added to each well and it was coated with a new plate sealer. Incubation was performed for about 15 minutes at 37℃. It might be protect the plate from light, and addition of 50µL from stop solution to each well.

**6.** Determination the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.

**Calculations:**

Average of the duplicate readings for each standard and samples. It was plotted a four-parameter logistic curve on log-log graph paper, with standard concentration on the x-axis and OD values on the y-axis. The concentration calculated from the standard curve must be multiplied by the dilution factor.

## 2.4.4 Determination of Serum Lipid Profile:

### 2.4.4.1 Determination of Serum Total Cholesterol (TC):

**Principle:**

Cholesterol esters are enzymatically hydrolyzed by cholesterol esterase to cholesterol and free fatty acids. Free cholesterol, including that primarily submits, is then oxidized by cholesterol oxidase to cholest4-ene-one and hydrogen peroxide. The hydrogen peroxide combines with hydroxyl benzoic acid (HBA) and 4-aminoantipyrine to form a chromophore (quinoneimine dye) which is quantitated at 500 nm [80].

**Preparation and Stability:**

One vial of reagent was reconstructed 2 with the appropriate volume of buffer/reagent 1. This working reagent is stable 4 months at 2 - 8°C or 1 month at 20 - 25°C.

**Procedure:**

| Pipette into tests tubes | | | |
|---|---|---|---|
| | Blank | Standard | Sample |
| Standard | - | 10 µl | - |
| Sample | - | - | 10 µl |
| Working reagent | 1 ml | 1 ml | 1 ml |
| Mix. incubate 5 mn. at 37°C before reading. The colour is stable for 30 mn. | | | |

Wave length: 505 nm (500-550) and Temperature: 37°C, Cuvette: 1 cm light path.

**Calculations:**

$$\text{Cholesterol} = \frac{\text{OD sample}}{\text{OD standard}} \times n$$

mmol/l: n = 5.17

**Reference Values:**

Serum or plasma: 3.6 - 7 mmol/l

Increased risk above: 6.7 mmol/l

## 2.4.4.2 Determination of Serum Triglyceride (TG):

**Principle:**

Triglycerides (TG) are enzymatically hydrolyzed by lipase to free fatty acids and glycerol. The glycerol is phosphorylated by adenosine triphosphate (ATP) with glycerol kinase (GK) to produce glycerol-3-phosphate and adenosine diphosphate (ADP). Glycerol-3-phosphate is oxidized to dihydroxyacetone phosphate (DAP) by glycerol phosphate

oxidase (GPO) producing hydrogen peroxide ($H_2O_2$). In a color reaction catalyzed by peroxidase, the $H_2O_2$ reacts with 4-aminoantipyrine (4- AAP) and 4-chlorophenol (4-CP) to produce a red colored dye. The absorbance of this dye is proportional to the concentration of TG present in the sample [81].

**Preparation and stability:**

Contents of one bottle of R2 were dissolved in the contents of one bottle buffer reagent R1. This working-reagent is stable for 4 weeks at 2 - 8°C 1 week at 20 - 25°C.

**Procedure:**

| | Blank | Standard | Sample |
|---|---|---|---|
| Standard | - | 10 µl | - |
| Sample | - | - | 10 µl |
| Working reagent | 1 ml | 1 ml | 1 ml |
| Mix, incubate 5 min. at 37°C or 10 min. at 25°C. The colour is stable for 30 minutes. | | | |

Wave length: 505 nm (490-550), Temperature: 37°C and Cuvette: 1 cm light path Read against blank

**Calculations:**

$$\text{TG Conc.} = \frac{\text{O.D.Sample}}{\text{O.D.standard}} \times n$$

mmol/l n = 2.28

Reference values:

Women: 0.46 - 1.60 mmol/l

Men: 0.68 - 1.88 mmol/l

## 2.4.4.3 Determination of Serum High Density Lipoprotein Cholesterol (HDL-C):

The Ultra HDL inspection is a homogeneous method for immediately gauge HDL-C concentration in serum or plasma without the want for off-line pretreatment or centrifugation proceedings. The method employs a two-reagent format and depends on the properties of a unique detergent. This method is based on accelerating the reaction of cholesterol oxidase (CO) with non-HDL unesterified cholesterol and dissolving HDL-C selectively employing a given detergent. In the former reagent, non-HDL unesterified cholesterol is subject to an enzyme reaction and the peroxide generated is consumed by a peroxidase reaction with disulfo butyl meta toluidine (DSBmT) yielding a colorless product. The latter reagent consists of a detergent (capable of solubilizing HDL-C), cholesterol esterase (CE), and chromogenic couple to improve color for the quantitative determination of HDL-C [82].

**Procedure:**

A solution of NaCI (9 g/l), serum and TG, more than 3,5mmol/l were dilute in the supernatant may be stored up to five days at 2 - 8°C.

**Cholesterol CHOD-PAP Assay:**

Pipette into the tubes:

| | Blank | Standard | Sample |
|---|---|---|---|
| Distilled water | 10µl | - | - |
| Standard (2 g/l) | - | 10µl | - |
| Supernatant | - | - | 10µl |
| Reagent solution | | | |
| cholesterol assay | 1 ml | 1 ml | 1 ml |

Wave length 500 nm, (492-550 nm), Cuvette: 1 cm light path, Temperature: 37°C and measurement: against reagent blank

Mix, incubate for 5 minutes at 37°C. Read against the reagent blank within 30 minutes.

## Calculations:

$$[\text{HDL-C}] = \frac{\text{OD measurement}}{\text{OD standard}} \times n$$

n = 5.17 mmol/l

Multiplying the result per 1.1 (1/11 is the dilution with the precipitant reagent) to obtain the concentration of cholesterol bound to HDL.

## Interpretation:

European Atherosclerosis Society has established relationship between risk level of coronary diseases and cholesterolemia.

<table>
<tr><th>Cholesterolemia</th><th colspan="2">Risk level</th></tr>
<tr><td>< 2 g/l<br>< 5,2 mmol/l</td><td colspan="2">Low risk</td></tr>
<tr><td>2,0 to 2,5 g/l<br>5,2 to 6,5 mmol/l</td><td>Moderate risk if</td><td rowspan="2">HDL<br>Cholesterol < 0,35 g/l<br>< 0,9 mmol/l)</td></tr>
<tr><td>> 2,5 g/l<br>> 6,5 mmol/l</td><td>High risk if</td></tr>
</table>

## 2.4.4.4 Determination of Serum Low and Very Low Density Lipoprotein cholesterol (LDL-C) and (VLDL):

The LDL-C and VLDL and the estimation of their concentrations are most commonly by Friedewald's equation for TG level less than 400 mg/dL [83].

LDL-C= TC - [ (TG)/5 + HDL-C], VLDL-C = TG (TG)/5

## 2.4.5 Determination of Renal Function Test:

## 2.4.5.1 Determination of Serum Urea:

A Serum urea test is employ to appreciation the quantity of urea in blood.

**Principle:**

The method is based on the pursue response:-

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

Salicylate and hypochlorite in the reagent react with the ammonium ions to form a green complex (2.2 dicarboxylindophenol) [84].

**Procedure:**

Pipette into test tubes:

| Tubes | Reagent blank | Standard | Sample |
|---|---|---|---|
| Standard | - | 10 µl | - |
| Sample | - | - | 10 µl |
| Working Reagent (R1) | 1000 µl | 1000 µl | 1000 µl |

The wave length 600 nm, Cuvette: 1 cm light path, Temperature: 25°C, 37°C and Measurement: against reagent blank

Mix. Incubate for at least 3 min at 37°C

| | | | |
|---|---|---|---|
| Sodium Hypochlorite (R2) | 200 µl | 200 µl | 200 µl |

Incubate for at least 5 min at 37°C. the absorbance of the criterion (A standard) and the absorbance of the specimen (Asample) against reagent blank within 2 hours.

## Calculations:

Urea concentration = $\frac{\text{A sample}}{A\ Standard} \times$ n

## Normal Values:

Serum: 2.5 - 7.5 mmol/l

## 2.4.5.2 Determination of Serum Creatinine:

**Principle:**

Creatinine in alkaline solution reacts with picric acid to form a coloured complex. The amount of the complex formed is directly proportional to the creatinine concentration [85].

**Procedure:**

Reaction rate and absorptivity of the reaction product are very sensitive to temperature. The specified temperature must therefore be maintained. A slight precipitate that may appear in solution R. It does not affect the assay.

**1.Deproteinization procedure:**

Pipette into centrifuge tubes:

The precipitated was mixed well using a glass rod. Then the participated was centrifuge at 2500 rpm for 10 min.

**2. Assay procedure :**

| | |
|---|---|
| Wavelength | 500-550 nm (Hg 546 nm) |
| Spectrophotometer | 520 nm |
| Cuvette | I cm light path |
| Temperature | 25℃ |
| Measurement | Against blank |

Pipette into test tubes:

| Tubes | Blank | Standard | Sample | Sample Urine |
|---|---|---|---|---|
| Distilled water | 0.5ml | - | - | - |
| Solution I (CAL) | - | 0.5ml | - | - |
| TCA | 0.5ml | 0.5ml | - | 0.5ml |
| Supernatant | - | - | 1.0ml | - |
| Urine (1+49) | - | - | - | 0.5ml |
| Reagent mixture | 1.0ml | 1.0ml | 1.0ml | 1.0ml |

Mix let stand for 20 min at 25℃. measure the absorbance of specimen (A sample) and criterion (A standard) against blank.

**Calculation**

Concentration of creatinine in serum(µmol/l) = $\frac{\boldsymbol{Asample}}{\boldsymbol{Astandard}}$× n

**Normal values:**

Men: 53-97 µmol/l

Women: 44-80 µmol/l

## 2.4.6 Determination of Serum Ferritin :

**Principle:**

The VIDAS Ferritin (FER) analyze was an enzyme linked fluorescent immunoassay (ELFA) performed in an automated device. each assay steps and assay temperature were controlled by the device. A pipette tip-like disposable device the Solid Phase Receptacle (SPR® ) serves as a solid phase for the analyze as well as a pipetting device. The (SPR) was coated at the time of manufacture with mouse monoclonal anti-ferritin antibodies. The VIDAS FER assay configuration prevents nonspecific reactions and SPR. Reagents for the analyze were located in the sealed Reagent Strips. The sample was transported into well containing the anti-ferritin antibody conjugated with alkaline phosphatase. The sample conjugate mixture was cycled in and out of (SPR) and the ferritin will bind to antibodies coated on SPR and to conjugate forming a (sandwich). Wash steps remove unbound conjugate. A fluorescent substrate 4-methylumbelliferyl phosphate was cycled through(SPR). Enzyme remaining on the (SPR) wall will catalyze the conversion of the substrate to the fluorescent produce 4-methylumbelliferone. The strength of fluorescence was measured by optical scanner in the device was proportional to ferritin concentration present in the sample. When the (VIDAS) Ferritin analyze was completed, the results were analyzed

automatically by instrument, and a report was printed for each sample[86].

**Procedure:**

1. The necessary components was removed from the kit and returned all unused components to storage at 2-8°C.

2. Allowing components to reach room temperature (approximately 30 minutes).

3. one FER strip was used for all sample control or calibrator to be tested. It should be the storage pouch has accurately resealed after the required SPRs have been removed.

4. The test is known by the FER code on the device. The calibrator might be identified by S1and tested in duplicate.

5. The FER reagent strips were labeled with the proper sample identification numbers.

6. The calibrator, control, and sample were mixed using a vortex type mixer (for serum separated from the pellet).

7. The calibrator, control, and sample test portion was 100 µl.

8. The FER reagent strips and SPRs were inserted into appropriate position on the device. It was checked the colors labels with the analyze code on the SPRs and the reagent strips match.

9. Initiation the analyze processing as directed in the operator's manual. every steps were executed automatically by the device.

10. The glass was reclosed and returned them to 2-8°C after pipetting.

11. The analyze was completed approximately 30 minutes. After the assay was completed the SPRs and strips were removed from the device.

12. The hired SPRs and reagent strips were disposed in aright recipient.

## 2.4.7 Determination of Serum Cortisol:

**Principle:**

VIDAS Cortisol is an automated assay for the VIDAS system, which enables cortisol in human serum or plasma to be directly quantitatively measured. The assay principle combines the enzymes immunoassay competition method with a final fluorescent discovery (ELFA). The Solid Phase Receptacle (SPR) serves as the solid phase as well as the pipetting machine. Reagents for the assay are preparedtouse and predisposed in the conserved reagent strips. All of the analyze steps are performed automatically by the device. The reaction medium is cycled in and out of the (SPR)a number of times. The taster is transferred into the well containing the conjugate: alkaline phosphatase-labeled cortisol derivative. The cortisol in the sample will compete with the cortisol derivative in the conjugate for sites on the specific anti-cortisol antibody which is fixed onto the interior of the SPR. Through the last detection step the substrate (4-Methylumbelliferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme stimulate the hydrolysis of this substrate into a fluorescent produce (4-Methylumbelliferone) the fluorescence of which is measurement at 450 nm. The strength of the fluorescence is inversely symmetric to the concentration of antigen present in the test. At the final of the assay the results are automatically calculated by the device in relation to the calibration curve stored in memory and then printed out [87].

**Assay procedure**

1. The required reagents were removed from the refrigerator.
2. One CORS section and one CORS SPR were used for all test control or calibrator to be tested. Make sure the storage sack has been carefully resealed after the required ( SPRs) have been removed.
3. The test was known by the CORS code on the device. The calibrator might identified byS1 and tested in triplicate.
4. The calibrator control and specimen (serum, plasma, extracted urine) were mixed using a vortex-type mixer.
5. For this test, the calibrator, control, and sample test portion is 100 µL.
6. The CORS SPRs and CORS strips were inserted into the instrument. The color labels were checked with the assay code on the SPRs and the reagent strips match.
7. Initiation the assay immediately. All the analyze steps were performed automatically by the device.
8. The vials and return were reclosed to 2-8°C after pipetting.
9. The assay was completed approximately 40 minutes. Then, the (SPRs) and strips were removed from the device.
10. The use (SPRs) and strips were disposed into an appropriate recipient.

## 2.4.8 Determination of Serum Testosterone:

**Principle:**

The evaluate principle combines an enzyme immunoassay competition process with a final fluorescent detection ELFA.

The Solid Phase Receptacle (SPR) serve as the solid phase as well as the pipetting device. Reagents for the evaluate are ready to use and predisposed in the conserved reagent strips.

All of the assay steps are performed willingly by the instrument. The reaction medium is cycled in and out of the SPR quite a few times.

The sample is supplement to the pre-treatment solution to detach the testosterone from transporter proteins.

The pre-treated sample was transfer into the fully containing an alkaline phosphates labeled testosterone antibody (conjugate). The antigen in the sample and the testosterone antigen limited to the interior dike of the SPR compete for the anti-testosterone specific antibody sites. Specified components are eliminated through washing steps.

During the final revealing step, the substrate (4-Methylumbelliferyl phosphate), is cycled in and out of the SPR the conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methylumbelliferone) the fluorescence of which is measured at 450 nm. The strength of the fluorescence is inversely proportional to the concentration of testosterone present in the sample. At the end of assay the results are automatically calculated by the instrument in relation to the calibration curve stored in memory and then printed out [88].

**Procedure :**

1. The required reagents were removed from the fridge and they can be used immediately.

2. The (TES) strip and one (TES) SPR were used for each sample control.

3. The test was identified by the (TES) code on the instrument. The calibrator might identified by (S1) and tested in repeat.

4. The samples were clarified by centrifugation.

5. The calibrator and , or the control and the samples were mixed using a vortex-type blender (for serum or plasma insular from the pellet).

6. Before pipetting, the samples calibrator and control should were free of bubbles.

7. For this test the calibrator control and sample test portion is 100µL.

8. The (TES) SPR and (TES) strips were inserted into the instrument.

9. Initiation the assay as directed in the User's Manual. All the assay steps were performed automatically by the instrument.

10. The flask was reclosed and returned them to the required temperature after pipetting.

11. The assay was completed within approximately 40 minutes. Then, the SPR and strips were removed from the instrument.

12. The free SPR and strip were received using the appropriate recipient.

**Normal Values:**

The measurement range of VIDAS Testosterone assay is: 0.05 to 13.50 ng/mL.

## 2.5 Data Processing and Statistical Analysis:

All statistical calculations were done using computer programs SPSS (Statistical Package of Social Science) program, version 17 software.
Data were expressed as means ± standard deviations (means ±SD); statistical significance was set at $p < 0.05$.

Comparison of numerical variables between the study groups was done using Student t-test for independent samples in comparing two groups at normally distributed.

Correlations among serum ghrelin and other parameters were analyzed by Pearson's correlations and multiple correlations analysis to determine the relationships between the variables of interest.

# Chapter three

## Results

## &

## Discussion

## 3.1 Age and BMI:

Results of Table (3-1) and Figure (3-1) show a highly significant increase in age in G2 and G3 as compared with G1.

Additionally, the result of BMI in Table (3-1) and Figure (3-2) revealed a substantial rise in G2 compared with G1 and G3.

**Table (3-1): Age and BMI in the study groups**

| Parameter | Means ± SD | | | *p*-value | |
|---|---|---|---|---|---|
| | G1 (n=50) | G2 (n=50) | G3 (n=50) | G1&G2 | G1&G3 |
| **Age (year)** | 47.79 ±8.34 | 57.68 ±7.01 | 54.48 ± 9.41 | HS | HS |
| **BMI (Kg/m$^2$)** | 25.60 ±3.84 | 32.21 ±4.07 | 27.61 ± 6.08 | S | NS |

G1: Control, G2: MI patients with DM, G3: MI patients without DM, HS: Highly significant, S: Significant, NS: Not significant.

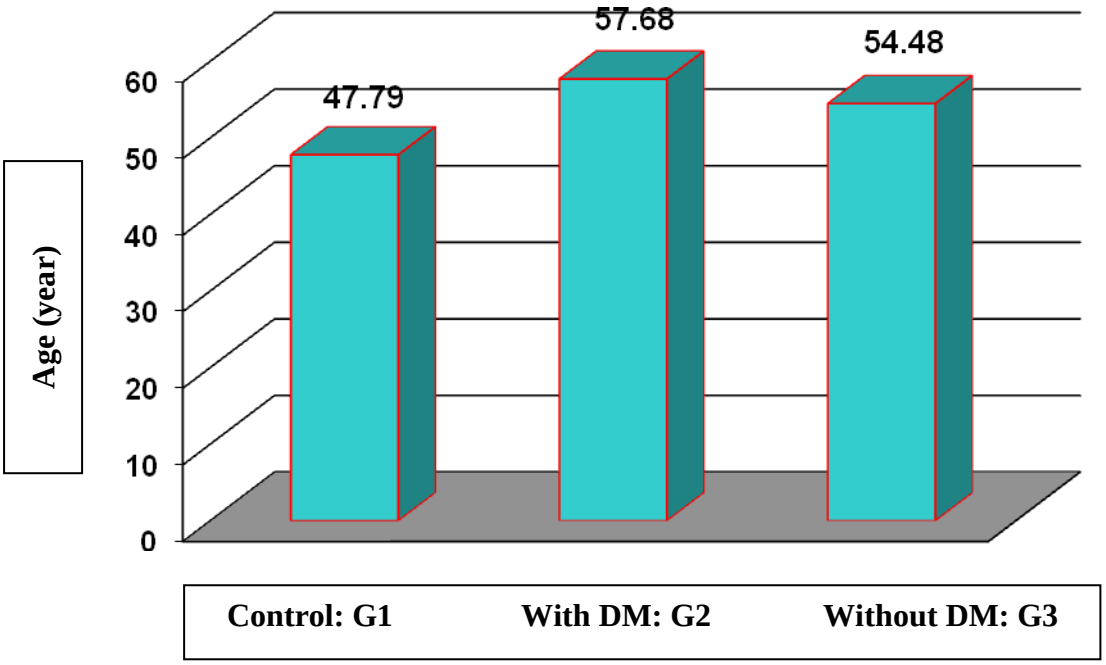


**Figure (3-1): Comparison between difference groups in age**

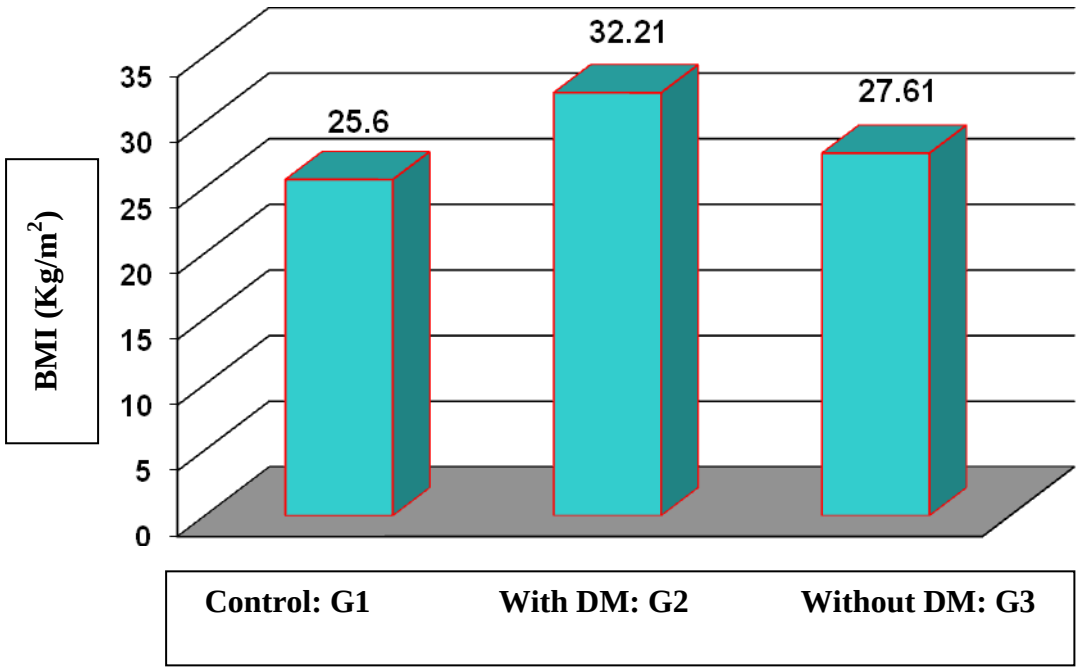


**Figure (3-2): Comparison between difference groups in BMI**

Individuals enrolled in this study between 40- 65 years old. This age group was designated in agreement with the nature of the disease formal, because T2DM was frequently well-defined as late-onset diabetes [89].

It has been postulated that there is a very strong association between DM and obesity. The increasing global incidence of T2DM is tied to growing rates of obesity [90].

Obesity is a condition in which there is a further of body fat contents the term means that the subject's body weight is greater than what's deliberated healthy for person's height. Body weight is a sum weight of body fat, bone, muscle, and body water. Obesity is the condition when BMI of equal or more than 30 $Kg/m^2$ [91], and the diabetic patients with MI in this study had BMI=32.21 ±4.07 $Kg/m^2$ .

Additional body fats accumulation is the principal cause of obesity. As obesity is related with various of physiological variations ensuing into

diseases such as IR, hypertension, and cardiac diseases. It has been documented a strong relationship of DM with obesity [92].

## 3.2 Fasting Serum Glucose, Insulin, and HOMA-IR:

Results of Table (3-2) and Figure (3-3) revealed a highly significant increase in FSG in G2 and G3 as compared with G1.

Result of serum insulin in Table (3-2) and Figure (3-4) denoted that there was a substantial increases in serum insulin in G2 as paralleled to G1, while there was no significant difference in serum insulin between G1 and. Additionally, there was a highly significant increase in HOMA-IR in G2 as compared to G1 and a significant increase in G3 as compared to G1, Figure (3-5).

**Table (3-2): The FSG, insulin, and HOMA-IR in the study groups**

| Parameter | Means ± SD | | | *p*-value | |
|---|---|---|---|---|---|
| | G1 (n=50) | G2 (n=50) | G3 (n=50) | G1&G2 | G1&G3 |
| **FSG (mmol/L)** | 5.06 ± 0.56 | 10.90 ±4.40 | 7.63 ± 2.83 | HS | HS |
| **Insulin (uU/ml)** | 10.29 ± 4.71 | 18.13 ±4.88 | 15.90 ±3.69 | S | NS |
| **HOMA-IR** | 2.82 ± 1.42 | 7.79 ± 4.92 | 5.71 ± 1.57 | HS | S |

G1: Control, G2: MI patients with DM, G3: MI patients without DM, HS: Highly significant, S: Significant, NS: Not significant.

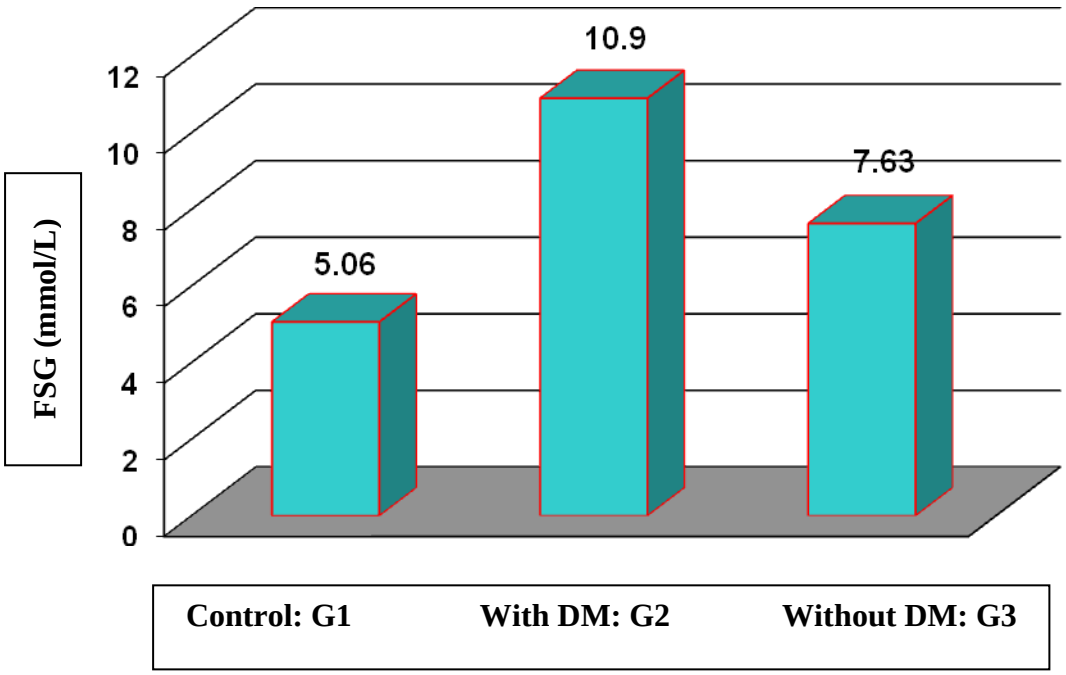


**Figure (3-3): Comparison between difference groups in FSG**

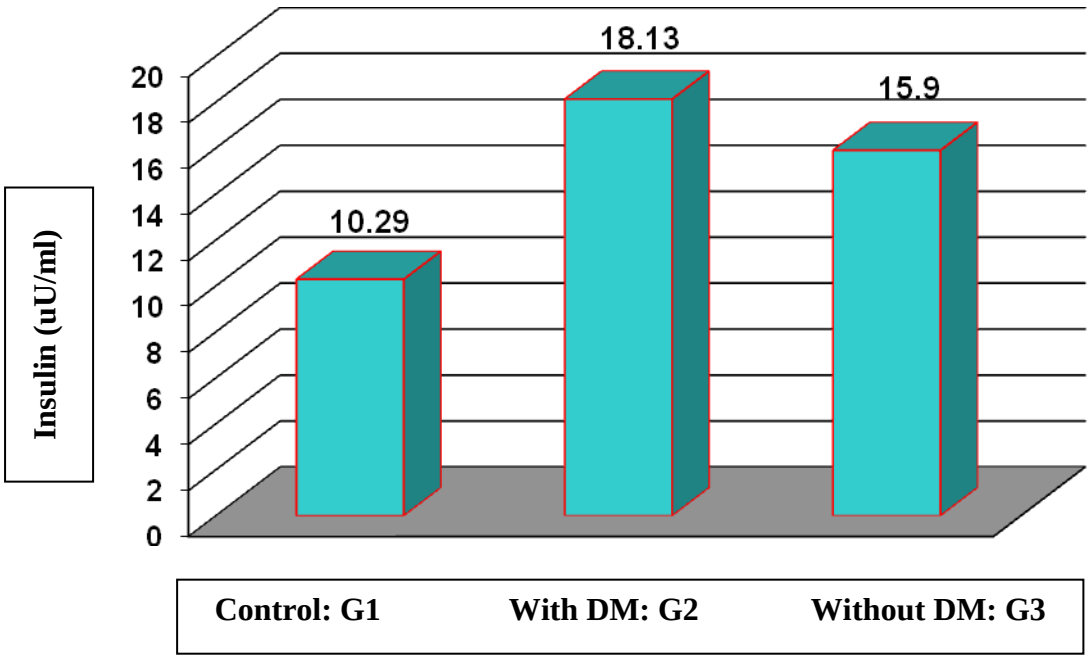


**Figure (3-4): Comparison between difference groups in insulin**

The MI patients with DM had the highly glucose levels. Numerous mechanisms of IR progress in diverse pathologic situations, including MI,

is a related concern [93, 94]. According to Nishio et al. (2006), IR in MI patients can help expect contrary disease result within 3 years of follow-up. Manifestation of IR in the initial phase of MI is one of the typical body responses to catecholamine stress.

In the current study, IR was diagnosed using traditional markers, i.e., serum glucose and insulin levels in addition to the IR index, which is in accordance with prior study [95].

Result of HOMA-IR in Table (3-2) and Figure (3-5) showed a significant increase in G2 as compared to G1 and a significant increase in G3 as compared to G1.

The increase in glucose, insulin, and HOMA-IR values were connected with greater risks of IR. This can be observed as a significance of pancreatic dysfunction and diminished β-adrenergic glucose metabolism regulation in hepatocytes through catecholamine stress [95].

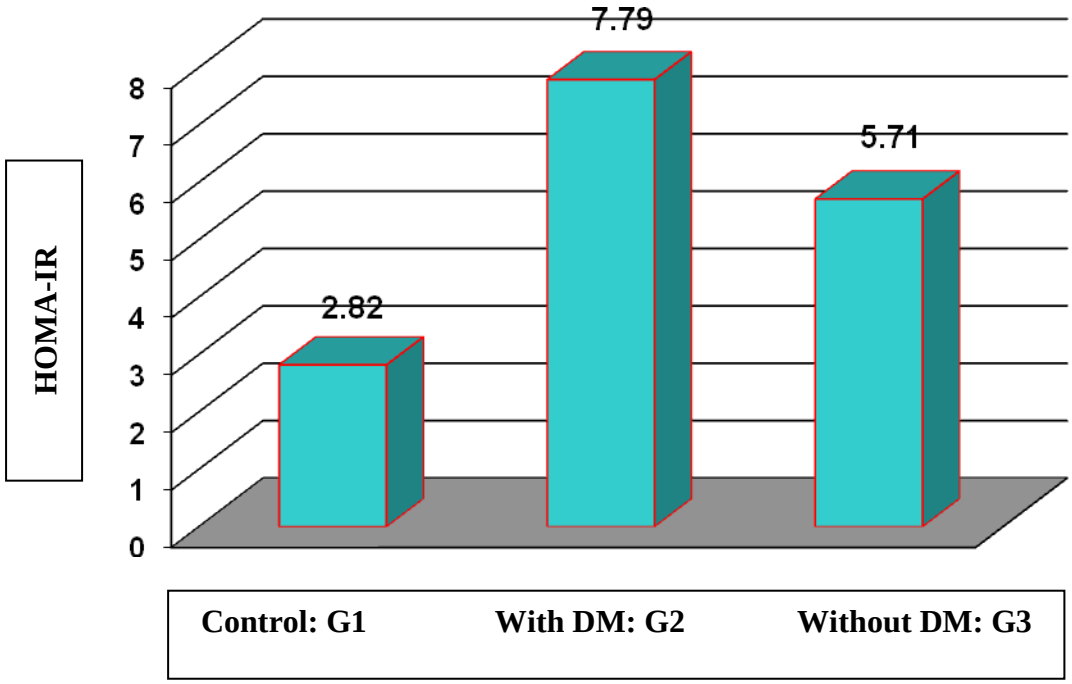


**Figure (3-5): Comparison between difference groups in HOMA-IR**

## 3.3 Serum Ghrelin:

Results of serum ghrelin in Table (3-3) and Figure (3-6) showed a highly substantial rise in serum ghrelin levels in G1 as paralleled to G2 and G3.

**Table (3-3): Serum ghrelin levels in the study groups**

| Parameter | Means ± SD | | | *p*-value | |
|---|---|---|---|---|---|
| | G1 (n=50) | G2 (n=50) | G3 (n=50) | G1&G2 | G1&G3 |
| **Ghrelin (ng/mL)** | 30.46± 7.54 | 19.15 ±4.77 | 21.19 ±4.09 | HS | HS |

G1: Control, G2: MI patients with DM, G3: MI patients without DM, HS: Highly significant, NS: Not significant.

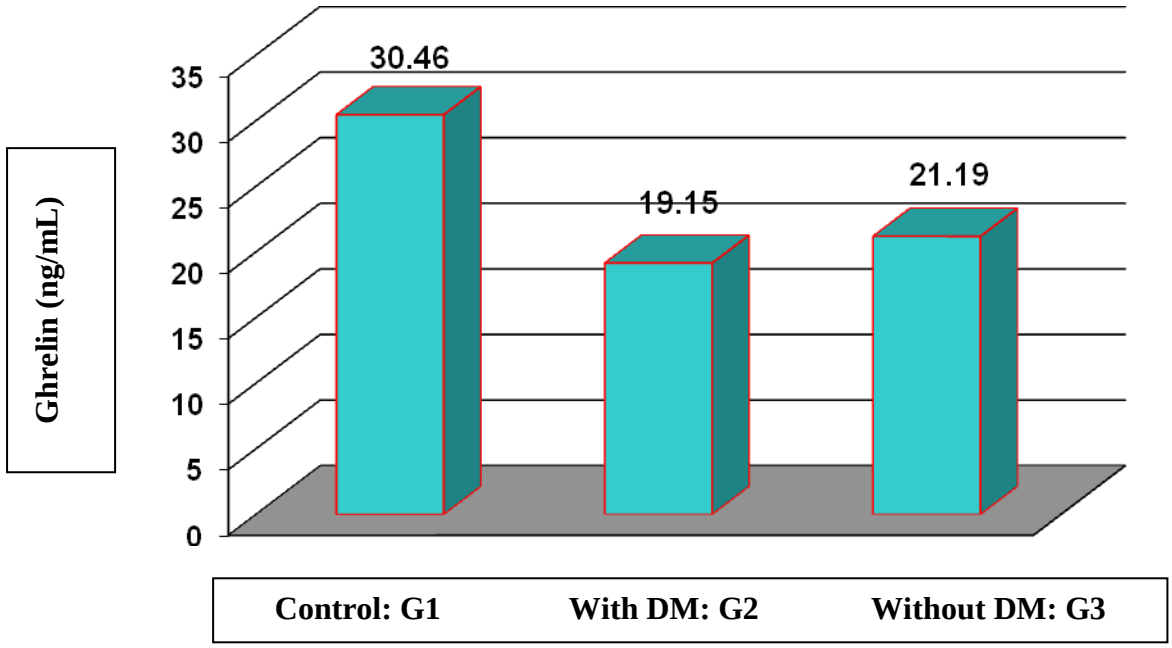


**Figure (3-6): Comparison between difference groups in Ghrelin**

The role of ghrelin, a gastrointestinal endocrine peptide and a significant regulator of growth factor secretion, desire for food and energy homeostasis. In IR pathogenesis has been deliberated recently [96]. It was found that cardiomyocytes are capable to yield ghrelin, which

has varied protective properties, comprising the inhibition of cardiomyocyte, endothelial cell apoptosis, and enhanced left ventricular function in ischemia/reperfusion [97].

Ghrelin is identified to modulate insulin secretion and, hence, is considered as a promising molecular IR marker. Ghrelin was revealed to donate to the expression of α- and β-insulin receptor subunits. At the same time, (1–10) nm/L of insulin suppress basal and noradrenaline stimulated ghrelin secretion but do not effect ghrelin mRNA expression [98]. Obese individuals have lesser ghrelin concentrations that those with normal metabolism; at the same time, ghrelin had a strong positive relationship with the HOMA-IR index regardless of anthropometric and metabolic limitations of IR syndrome [99].

In the current study, ghrelin concentrations were considerably reduced in MI patients during the entire hospital stay, also in patients with IR, the alterations were more distinct. It was revealed that MI patients have reduced ghrelin levels, may due to an improved binding of ghrelin with its receptor in ischemia/reperfusion [100]. It has been proposed that in MI, the inhibition of ghrelin secretion may also be due to the inequity in the adipokine system accompanied by the dysfunction of insulin-secreting pancreatic cells, impaired lipid metabolism and IR manifestation [101]. Furthermore, the ghrelin level seems to be a more revealing IR marker than traditional markers and adipokine status factors in both the acute and recovery MI phases. Ghrelin has an improved diagnostic value than insulin in the acute MI phase [102].

This study found that ghrelin levels in type 2 diabetic patients were considerably less than those of healthy group which was in line with studies [103, 104].

The present study outcomes support the opinion that decrease ghrelin concentration might have a contributory role in the progress of T2DM. Additionally, the current data were also definite by an earlier revision which showed that decreased ghrelin concentrations are independently related with IR and T2DM [105]. On the other hand, these results were not definite by Shiiya et al., 2002 [106]work who found that ghrelin levels of type 2 diabetic patients were not diverse from controls and ghrelin concentrations appeared to fluctuate rendering to the BMI and not by diabetes. One study showed that obese individuals with T2DM had lesser ghrelin levels than lean individuals with T2DM. This designated that ghrelin did not seem to be connected with glucose or insulin metabolism, thus the liberated role of BMI, glucose and insulin in ghrelin regulation is still debated [107].

These outcomes propose that ghrelin shows both physiological and pathophysiological roles in insulin release that should be additional examined. On the other hand, current data was not sustained by other workers who revealed a negative relationship between total ghrelin and insulin levels. Additionally, various previous revisions revealed that ghrelin has been shown to inhibit insulin release in some investigational states [108, 109].

## 3.4 Serum Lipid Profile:

Result of serum lipid profile levels in Table (3-4), Figures [(3-7)-(3-11)] showed a highly significant increase in serum TC and TG in G2 and G3 as compared with G1. Also, there was a significant decrease in serum HDL-C in G2 and G3 as compared with G1.

Moreover, there was a substantial rise in serum LDL-C and VLDL in G2 as paralleled to G1 and G3.

**Table (3-4): Serum lipid profile in the study groups**

| Parameter | Means ± SD | | | *p*-value | |
|---|---|---|---|---|---|
| | G1 (n=50) | G2 (n=50) | G3 (n=50) | G1&G2 | G1&G3 |
| **TC (mmol/L)** | 4.62 ± 0.38 | 6.02 ± 1.44 | 5.74 ± 1.45 | HS | HS |
| **TG (mmol/L)** | 1.27 ± 0.26 | 2.06 ± 0.74 | 1.83 ± 0.40 | HS | HS |
| **HDL-C (mmol/L)** | 1.68 ± 0.28 | 1.21 ± 0.26 | 1.21 ± 0.39 | S | S |
| **LDL-C (mmol/L)** | 3.43 ± 0.54 | 4.40 ± 1.51 | 4.16 ± 1.47 | HS | S |
| **VLDL (mmol/L)** | 0.255 ±0.05 | 0.412 ±0.15 | 0.366 ±0.08 | HS | HS |

G1: Control, G2: MI patients with DM, G3: MI patients without DM, HS: Highly significant, S: Significant, NS: Not significant.

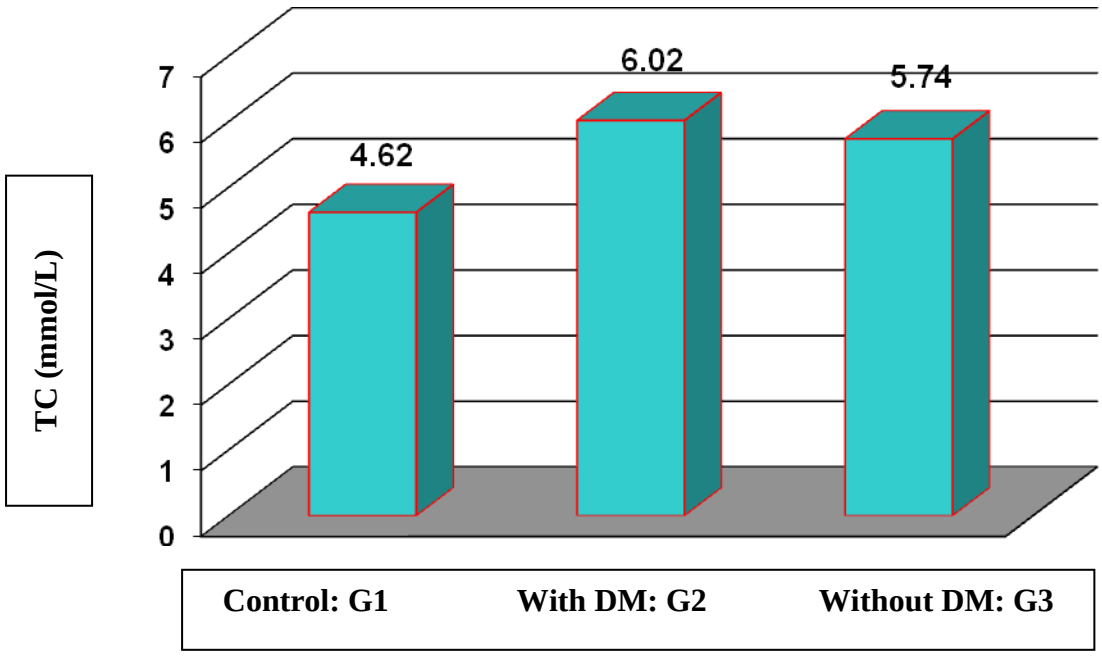


**Figure (3-7): Comparison between difference groups in serum TC**

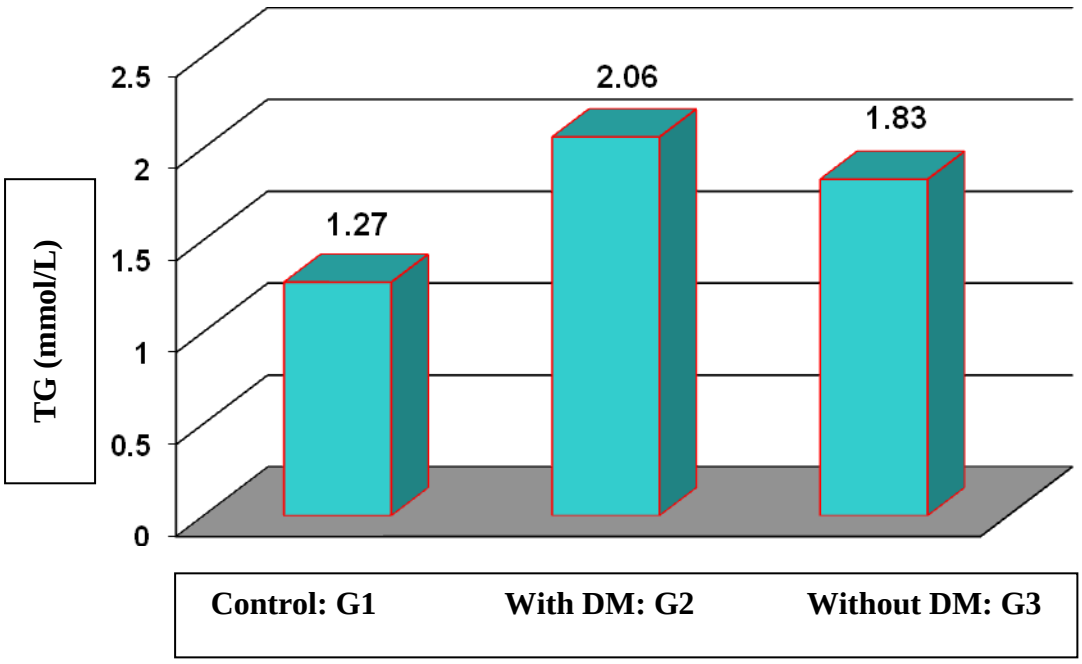


**Figure (3-8): Comparison between difference groups in serum TG**

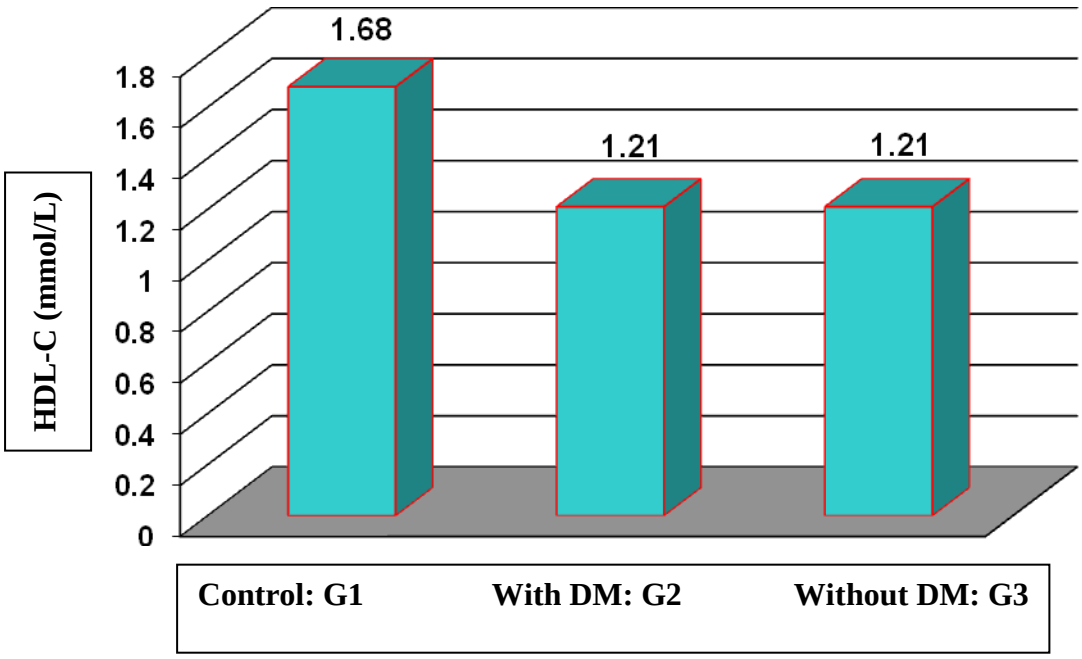


**Figure (3-9): Comparison between difference groups in serum HDL**

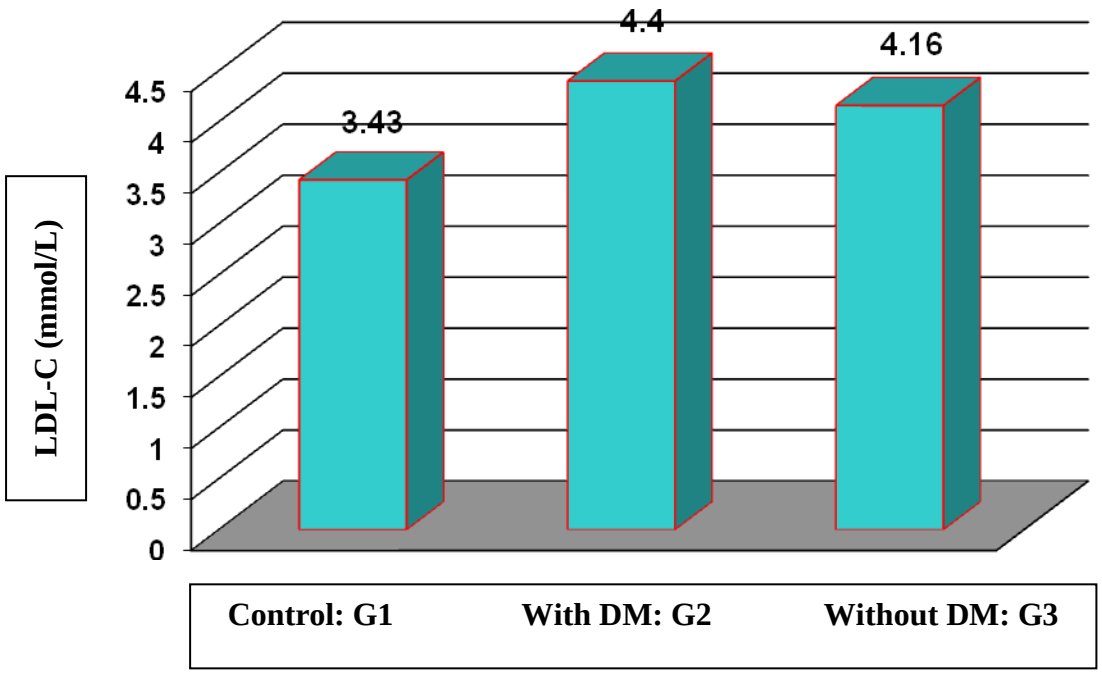


**Figure (3-10): Comparison between difference groups in serum LDL-C**

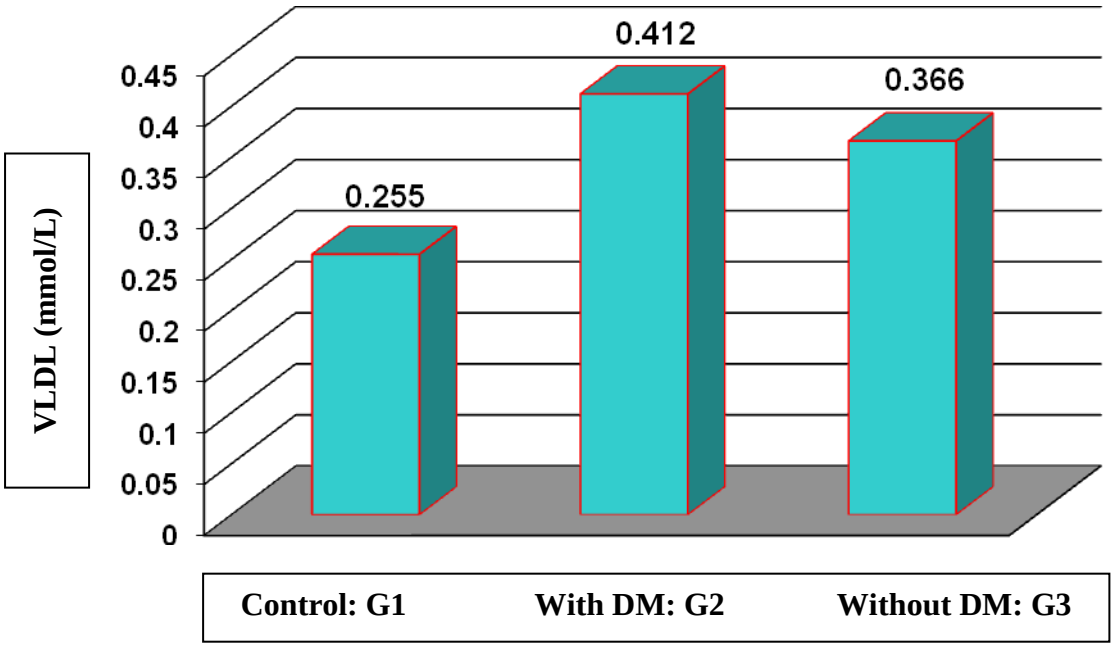


**Figure (3-11): Comparison between difference groups in serum VLDL**

Dyslipidemia is one of the important diabetic complications which is a classical risk factor for cardiovascular disease [110]. The mechanisms by which hyperglycemia and dyslipidemia cause diabetic vascular diseases are the formation and accumulation of advanced glycation end products (AGEs), improved oxidative stress, triggering of protein kinase C (PKC), elevated flux through the hexosamine pathway, vascular inflammation, deficit of insulin action in the vasculature, and modified growth factors, expression and action of hormones, and cytokines [111]. Furthermore, chemical alteration of lipoprotein in diabetic situations, including glycation and peroxidation, may be a causal pathogenic mechanism associating dyslipidemia to diabetic complications. For example, glycation may enhance the oxidative stress of the lipoproteins while oxidation may enhance atherogenicity of the lipoproteins. Moreover, chemical alteration of proteins by lipids, such as formation of lipoxidation end products, has also been proposed to be a prospective pathogen for vascular alterations in diabetics [112].

In this study, there was a substantial increase in serum lipid profile, except serum HDL-C which was significantly decreased in MI patients with DM and this is in accordance with the study of Bhattacharjee et al., 2018 [113].

The IR in MI patients was related to a gathering of CV risk factors, comprising obesity, dyslipidemia, and hypertension, described by elevated VLDL and TG levels and lesser HDL-C levels, which is in accordance with previous data [114].

The high glucose level detected in diabetics triggers the polyol (sorbitol-aldose reductase) pathway, which leads to intracellular sorbitol accumulation. The inability of sorbitol to pass through the cell membrane

in insulin-independent tissues (the nervous system, retina, and kidney) causes an elevated in intracellular osmotic pressure and, consequently, cell damage. In oxidative stress conditions, all intermediates of the polyol pathway (sorbitol, fructose, and fructose-1-phosphate) can glycate proteins leading to AGE formation, and this is concerned in microvascular complications of DM [115]. Interestingly, an elevation in glucose and glycogen levels detected under caloric restriction situations has been found to cause the substantial reduction in the activities of the polyol pathway enzymes, along with the activation of hexokinase, glucose-6-phosphate-dehydrogenase (pentose phosphate pathway enzyme), and glucose-6-phosphatase (glycogen degradation enzyme) in both diabetic and non-diabetic rats. Consequently, caloric restriction donates to the attenuation of hyperglycemia detected in DM. The formation of AGEs has been described to associate with glycemic control [116].

The accumulation of AGEs increasingly leads to cellular dysfunction and tissue damage complicated in the progress of other oxidative stress-induced chronic diseases such as atherosclerosis and DM. Hyperglycemia can induce oxidative stress and tissue damage through either repeated acute alterations in glucose metabolism or long-term biomolecular glycation and AGE formation [117]. This can further activate inflammation and cell proliferation contributing to the progress of atherosclerosis and vascular dysfunction through the initiation of oxidation of (low density lipoprotein particles) LDLs and their interaction with mononuclear cells, endothelial cells, and smooth muscle cells [118]. Glycation of LDLs increases their atherogenicity, while HDLs have been assumed to inhibit the glycation of LDL apolipoprotein B (apoB). In an

atherosclerotic lesion, macrophages express scavenger receptors on the surface of their cell membrane to bind oxidized LDLs from blood vessel walls and to progress into foam cells. The oxidation of LDLs causes the formation adducts that donate to the atherogenicity of LDLs and their binding capacity to scavenger receptors [119]. Furthermore, the transportation of oxidized lipids in lipoprotein complexes has been proposed to play a role in the pathogenesis of atherosclerosis, those transported by LDL being associated with high risk, while those transported by HDL being suggestive for defense against disease progression [120].

The main disorders of lipid metabolism in the current study are hypercholesterolemia and hypertriglyceridemia. Elevated concentrations of serum TG has been associated with an elevated LDL particles and improved CV risk had a strong association with the diameter of LDL-C particle, suggesting it as an indirect index for the diameter of LDL-C particle, LDL-C particles become smaller and denser, which contribute to the development of atherosclerosis and CVD. This finding is in accordance with a study [121].

Diverse values of serum lipid profile in some studies may be owed to diverse nutritional habits among obese, inactive, or high dietary fat–intake, related with the non-obese diabetic and control groups [122, 123]. These outcomes are consistent with the present study.

Though, usually, desirable cholesterol levels are up to 180 mg/dl for TC, up to 100 mg/dl for LDL-C, and not less than 50 mg/dl for HDL-C. When serum cholesterol concentrations exceed these levels, it is referred to as hypercholesterolemia [124].

## 3.5 Serum Urea and Creatinine:

Result of serum urea levels in Table (3-5) and Figure (3-12) showed a high significant increase in serum urea in G2 and G3 as compared with G1.

Additionally, the result of creatinine levels showed a significant increase in serum creatinine in G2 and G3 as compared with G1,Figure (3-13).

**Table (3-5): Serum urea and creatinine levels in the study groups**

| Parameter | Means ± SD | | | *p*-value | |
|---|---|---|---|---|---|
| | G1 (n=50) | G2 (n=50) | G3 (n=50) | G1&G2 | G1&G3 |
| **Urea (mmol/L)** | 4.97 ± 1.50 | 8.04 ± 2.92 | 7.20 ± 3.79 | HS | HS |
| **Creatinin (mmol/L)** | 78.10 ± 10.92 | 102.62 ± 56.63 | 99.49 ± 51.78 | S | S |

G1: Control, G2: MI patients with DM, G3: MI patients without DM, HS: Highly significant, S: Significant, NS: Not significant

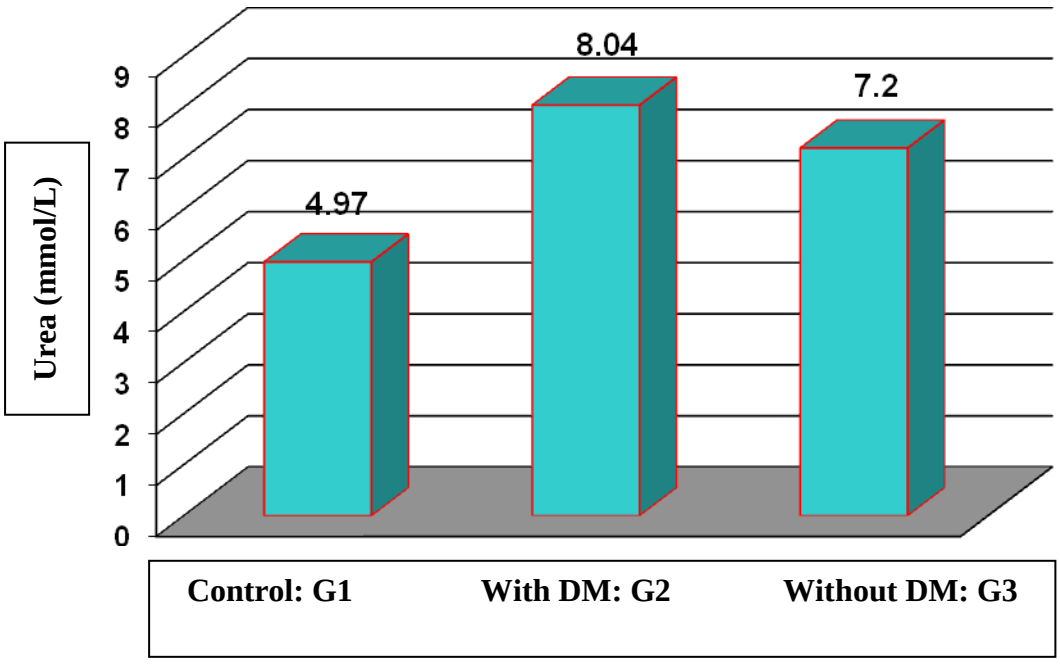


**Figure (3-12): Comparison between difference groups in serum urea**

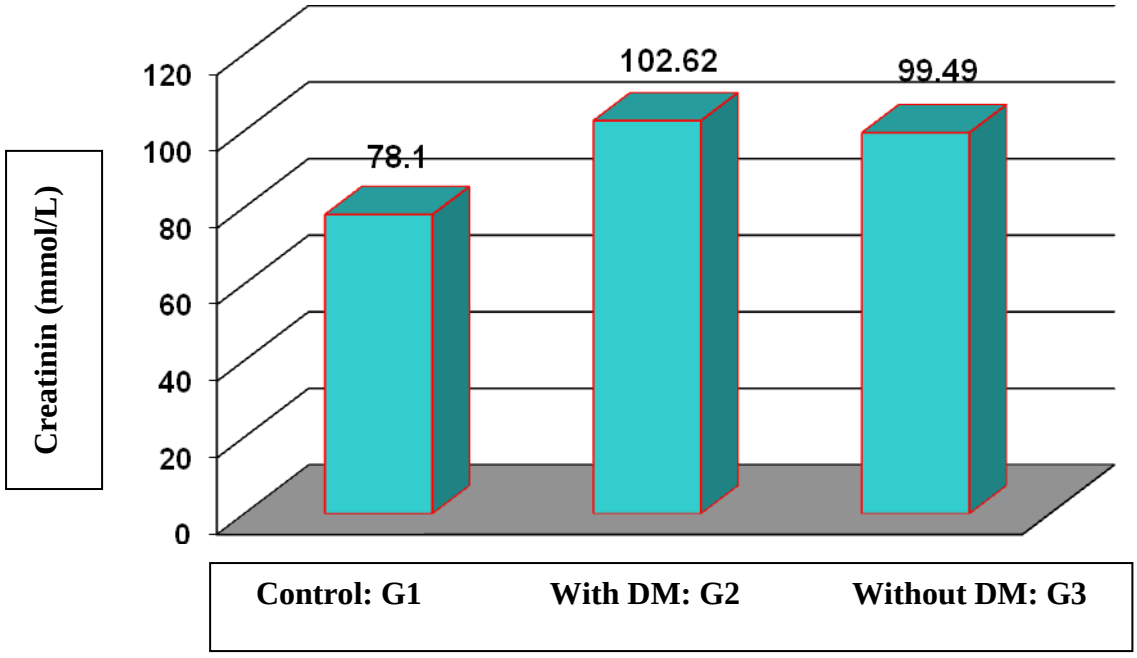


**Figure (3-13): Comparison between difference groups in serum creatinine**

Blood urea and creatinine have a better guiding value for the clinical treatment of AMI patients. Elevated of their values are related with an improved risk of long-term mortality in patients with AMI [125, 126]. Both the renin-angiotensin-aldosterone system (RAAS) and sympathetic nervous system (SNS) were triggered in patients with CVD, which could promote absorption of water and sodium and cause passive reabsorption of blood urea in the renal tubules [127].

Activation of the neurohormonal system could also lead to renal vasoconstriction and reduced glomerular filtration rate and BUN excretion [128]. Additionally, inadequate blood volume secondary to low cardiac output stimulates the release of arginine vasopressin (AVP), which can expedite the reabsorption of blood urea in the collecting duct. For the causes that the creatinine was freely filtered through the glomerulus and not reabsorbed [129].

For patients with AMI, higher blood urea and creatinine reflect a more active neurohormonal system. It is generally supposed that activation of the RAAS and SNS systems is related to contrary prognosis [130, 131].

Elevated levels of protein intake, i.e. > 1.3 g/ kg/day, are related to increased albuminuria, more rapid defeat of renal function and increased CV mortality in diabetic patients. Decreasing protein intake below 0.8 g/kg/ day is unlikely to slow renal decay or decrease CV risk and is not suggested [132]. It is not identified if a low protein diet reduces the risk of developing diabetic kidney diseases (DKD). In general, dietary patterns that are high in plant and sea foods and low in processed foods,

e.g. the Mediterranean diet, are more likely to slow development of DKD [133].

Diabetic patients are at improved risk of acute kidney injury (AKI), paralleled to those without DM, and the risk is even greater in those with AKI. Approximately 10% of patients hospitalized with AKI need kidney replacement therapy and mortality rates of 50% are reported for patients with this severity of injury [134].

## 3.6 Serum Ferritin:

Results of serum ferritin in Table (3-6) and Figure (3-14) showed a highly substantial increase in serum ferritin in G2 as compared with G1, and also showed a significant increase in G3 as compared to G1.

**Table (3-6): Serum ferritin levels in the study groups**

| Parameter | Means ± SD | | | *p*-value | |
|---|---|---|---|---|---|
| | G1 (n=50) | G2 (n=50) | G3 (n=50) | G1&G2 | G1&G3 |
| **Ferritin (ng/ml)** | 117.48 ±48.43 | 209.79 ±98.82 | 189.31 ±68.90 | HS | S |

G1: Control, G2: MI patients with DM, G3: MI patients without DM, HS: Highly significant, S: Significant, NS: Not significant.

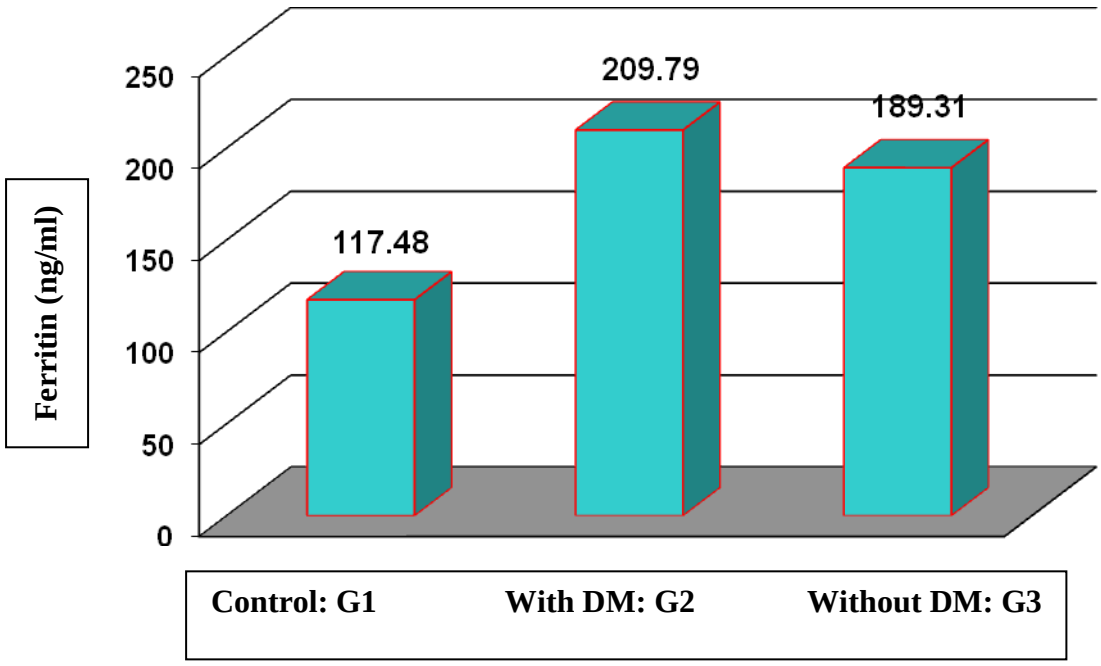


**Figure (3-14): Comparison between difference groups in serum ferritin**

A growing body of indication has proposed that excessive values of ferritin, either low or high, may be prognostic of improved mortality in patients with metabolic, renal, CV, hematological, neoplasmatic, and other diseases. Further population revisions have delivered weak indication of such relationship among people without medical history of chronic diseases [135].

In men, most studies have revealed either positive or no association between circulating ferritin and CV mortality independent of other contributing factors [136, 137]. However, the overall interaction between serum ferritin levels and CV mortality appears to be more multifaceted. Men without pre-existent chronic diseases and high ferritin levels (194-598 ng/ml) seemed with increased CV death risk independent of confounders. This has a clinical implication, since measured ferritin levels higher from reference spectrum may improve substantial value in CV prognosis in otherwise healthy men [138].

Other revisions proposed a relationship among women, between serum ferritin and CV mortality and morbidity [139]. In this studies, MI patient with DM had higher ferritin levels so, they had higher all-cause and CV mortality risk paralleled to those in the reference quartile. These relationships persisted unaffected after adjustment for covariates comprising anemia and inflammatory markers. Subgroup analysis also confirmed poor prediction in women with low serum ferritin in the absence of baseline chronic diseases.

The mechanism underlying the sex-specific relationships between circulating ferritin and mortality is expected to be multifaceted, and may not simply reveal alterations in body iron stores with obvious clinical consequences. Apparently, males and females respond differently to iron-induced myocardial injury, as this has been concerned in an investigational study [140].

In comparison, higher serum ferritin concentrations (hyperferritinemia) in MI patients with and without DM have been considerably associated with higher risk of metabolic diseases such as DM, obesity, and hyperlipidemia with subsequent increase of CV risk [141].

Moreover, inflammatory processes may effect circulating ferritin levels. In the context of CVD, ferritin may utilize a dual pro-inflammatory role. Its higher concentrations may denote an acute phase reactant consistently to high sensitivity c-reactive protein (hsCRP), while the reduced concentration may also precipitate inflammation. Therefore, both high and low ferritin concentrations may relate to improved inflammation, prompting the development of CVD and other chronic diseases [142].

The present data may conflicts the multi-practical role of ferritin, hypothetically complicated in other non-inflammatory pathways like angiogenesis.

## 3.7 Serum Cortisol:

Results of serum cortisol in Table (3-7) and Figure (3-15) showed a highly substantial rise in serum cortisol in G2 and G3 as paralleled with G1.

**Table (3-7): Serum cortisol levels in the study groups**

| Parameter | Means ± SD | | | *p*-value | |
|---|---|---|---|---|---|
| | G1 (n=50) | G2 (n=50) | G3 (n=50) | G1&G2 | G1&G3 |
| **Cortisol (ng/ml)** | 88.17 ± 18.76 | 145.62 ±10.16 | 141.93 ± 38.26 | HS | HS |

G1: Control, G2: MI patients with DM, G3: MI patients without DM, HS: Highly significant, NS: Not significant.

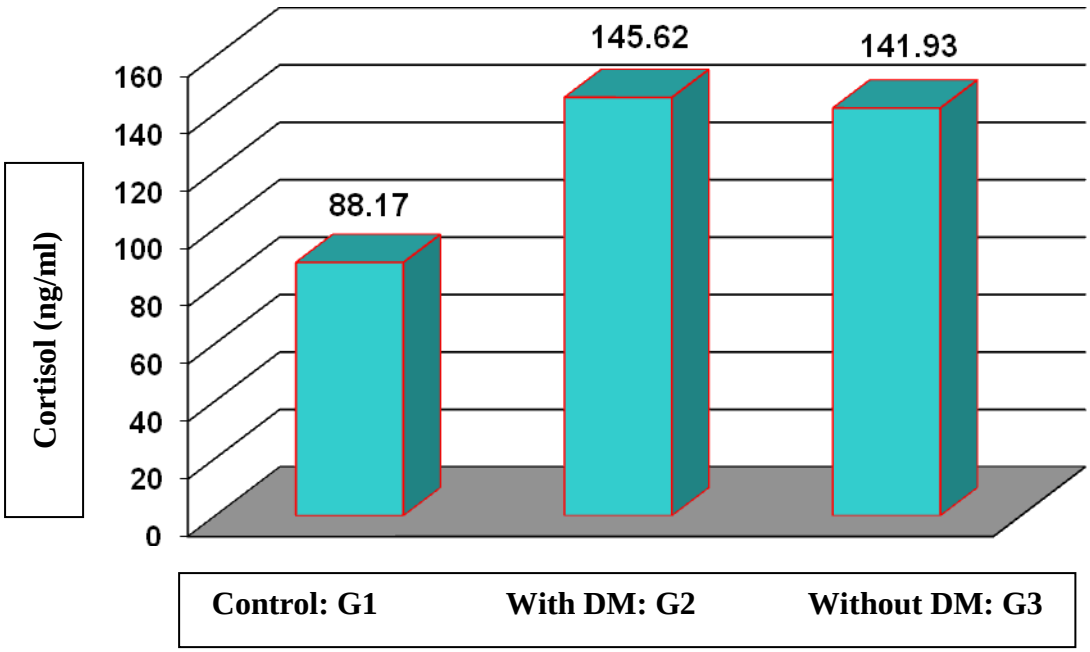


**Figure (3-15): Comparison between difference groups in serum cortisol**

It has been reported that morning plasma levels of cortisol to be positively associated with plasma glucose, blood pressure, and other CV risk factors [143].

Associations have also been found between cortisol and markers of sub-clinical atherosclerosis including carotid plaques and CA calcification. Evidence of an association between circulating cortisol levels and CVD events is, however, less conclusive, with studies to date mostly being cross-sectional [144]. Study of Bowden et al., 2016 has reported a positive association between morning plasma cortisol and CVD, but effects were imprecisely estimated due to the small study sizes (number of incident CVD cases between 63 and 320) [145].

Large study analysis uses the genetic predictors of morning plasma cortisol as instrumental variables in Mendelian randomization analyses, has found that blood cortisol is a causal risk factor for CVD [146].

This study suggests that the elevated morning serum cortisol that is associated with CVD is may be attributed to a response of the hypothalamic-pituitary-adrenal axis to chronic inflammation of subclinical CV.

## 3.8 Serum Testosterone:

Results of serum testosterone in Table (3-8) and Figure (3-16) showed a reduction in serum testosterone in G2 and G3 as paralleled to G1, but it was not substantial.

**Table (3-8): Serum testosterone levels in the study groups**

| Parameter | Means ± SD | | | *p*-value | |
|---|---|---|---|---|---|
| | G1 (n=50) | G2 (n=50) | G3 (n=50) | G1&G2 | G1&G3 |
| **Testosterone (ng/ml)** | 1.61 ± 1.46 | 1.32 ± 1.08 | 1.54 ±1.48 | NS | NS |

G1: Control, G2: MI patients with DM, G3: MI patients without DM, NS: Not significant.

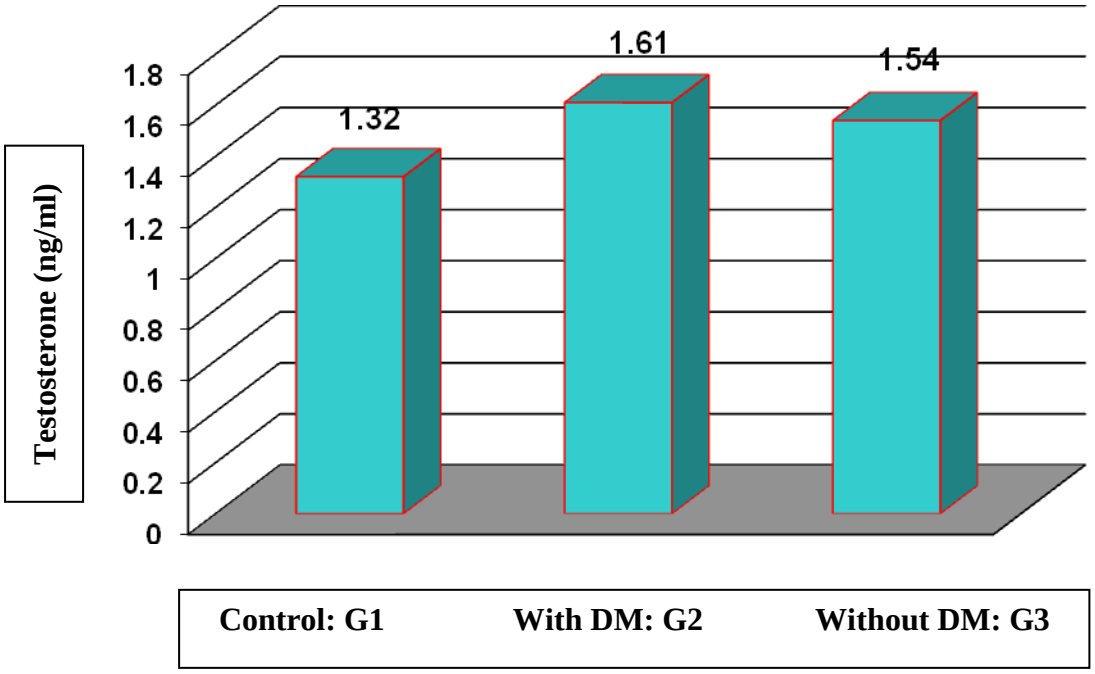


**Figure (3-16) Comparison between difference groups in serum testosterone**

This hormone has essential physiological roles and deficit can harmfully affect the fat, bone, muscle, brain, peripheral nerves, CV system and particularly the male genital and reproductive systems [147]. Testosterone is vital for the regulation of carbohydrate metabolism, lipids and proteins, and positively disturbs glucose control, liver fat, cardiac biomarkers, muscle growth and adipogenesis [148].

Araujo et al.,2011 achieved a meta-analysis that involved 18 studies and more than 22,000 subjects and concluded that both overall and CV mortality were associated with testosterone levels. Testosterone therapy

in hypogonadal men intervals time to ischemia, advances mood and is related with hypothetically beneficial reductions in biomarkers, TC, and serum tumor necrosis factor-alpha [149].

It has been revealed that administration of testosterone progresses myocardial ischemia in men with CVD. This trial found that in men with CVD, testosterone delays the time to exercise-induced ST-segment depression as measured on treadmill stress testing [150].

Additionally, a negative relationship it has been documented between endogenous testosterone levels and intima-media thickness of the carotid arteries, abdominal aorta and thoracic aorta, which proposes that men with reduced levels of endogenous testosterone may be at a greater risk of evolving more generalized atherosclerosis [151].

Evidence also proposes that men with reduced testosterone levels are more expected to progress CVD during their lifetime and CVD severity has been revealed to associate with the degree of testosterone deficit [152].

Daka et al.,2015 reported that reduced concentrations of testosterone expected acute MI in men with T2DM [153], which is in agreement with the present study.

Yeap et al.,2014 studied 3,690 older men over 10 years, total- and free- testosterone levels in the normal value were related with decreased all-cause and CV mortality. Interestingly, both low and high levels of testosterone were related with all-cause mortality, and elevated levels of dihydrotestosterone decreased ischemic heart disease mortality [154].

A review by Muraldeedharan et al.,2015 documented that there is no pathogenic link, but low testosterone may merely be a marker of disease [155].

## 3.9 Correlations among Study Analysis:

**Table (3-9): Correlation coefficient (r) between serum ghrelin and other studied parameters**

| Serum Ghrelin ng/mL | | $G_2$ | $G_3$ |
|---|---|---|---|
| **Age** | **R** | 0.21 | 0.09 |
| | **P** | 0.120 NS | 0.460 S |
| **BMI $Kg/m^2$** | **R** | -0.08 | -0.05 |
| | **P** | 0.517 S | 0.691 S |
| **FSG mmol/L** | **R** | -0.10 | -0.11 |
| | **P** | 0.449 NS | 0.394 NS |
| **Insulin uU/ml** | **R** | -0.06 | -0.003 |
| | **P** | 0.678 S | 0.984 HS |
| **HOMA-IR** | **R** | -0.02 | -0.07 |
| | **P** | 0.853 HS | 0.585 S |
| **TC mmol/L** | **R** | -0.14 | -0.15 |
| | **P** | 0.296 NS | 0.255 NS |
| **TG mmol/L** | **R** | -0.25 | -0.10 |
| | **P** | 0.066 NS | 0.439 S |
| **HDL-C mmol/L** | **R** | 0.11 | 0.15 |
| | **P** | 0.385 S | 0.230 NS |
| **LDL-C mmol/L** | **R** | -0.19 | -0.19 |
| | **P** | 0.135 NS | 0.149 NS |

| | | | |
|---|---|---|---|
| **VLDL mmol/L** | **R** | -0.25 | -0.10 |
| | **P** | 0.066 NS | 0.439 S |
| **Urea mmol/L** | **R** | -0.24 | -0.14 |
| | **P** | 0.071 NS | 0.447 S |
| **Creatinine µmol/L** | **R** | -0.48 | -0.29 |
| | **P** | 0.0001 NS | 0.032 NS |
| **Ferritin ng/ml** | **R** | -0.04 | -0.13 |
| | **P** | 0.713 HS | 0.279 NS |
| **Cortisol ng/ml** | **R** | -0.07 | -0.01 |
| | **P** | 0.573 S | 0.921 HS |
| **Testosterone ng/ml** | **R** | 0.41 | 0.15 |
| | **P** | 0.0012 NS | 0.262 NS |

## 3.9.1 Correlation of Serum Ghrelin and Age:

There was a positive correlation between serum ghrelin and age (r= 0.21, *p= 0.*120), (r= 0.9, *p*= 0.460) in $G_2$ and $G_3$ respectively, but it was not significant as revealed in Table (3-9).

## 3.9.2 Correlation of Serum Ghrelin and BMI:

There was a negative correlation between serum ghrelin and BMI (r= -0.08, $P \geq 0.05$), (r= -0.05, $P \geq 0.05$) in $G_2$ and $G_3$ patients respectively as revealed in Table (3-9).

### 3.9.3 Correlation of Serum Ghrelin and FSG:

There was a negative correlation between serum ghrelin and FSG in $G_2$ and $G_3$ (r= 0.10, P ≥ 0.05), (r= 0.11, P ≥ 0.05) in $G_2$, $G_3$ patients and respectively as revealed in Table (3-9).

### 3.9.4 Correlation of Serum Ghrelin and Insulin:

There was a negative correlation between serum ghrelin and insulin (r = -0.06, P ≥ 0.05) in $G_2$, and (r= - 0.003, P ≥ 0.05) in G3, but they were not significant as revealed a Table (3-9).

### 3.9.5 Correlation of Serum Ghrelin and HOMA-IR:

There was a negative correlation between serum ghrelin and HOMA-IR (r= 0.02, P ≥ 0.05) in G2 and (r= - 0.07, P ≥ 0.05) in G3 as revealed in Table (3-9).

### 3.9.6 Correlation of Serum Ghrelin and TC:

There was a substantial negative correlation between serum ghrelin and TC (r= -0.14, P ≥ 0.05) in $G_2$, and (r= - 0.15, P ≥ 0.05) in $G_3$ patients, as revealed in Table (3-9).

### 3.9.7 Correlation of Serum Ghrelin and TG:

There was a negative correlation between serum ghrelin and TG (r= 0.25, P ≥ 0.05) in G2, and (r= 0.10, P ≥ 0.05) in G3 as revealed in Table (3-9).

### 3.9.8 Correlation of Serum Ghrelin and HDL-C:

There was a positive correlation between serum ghrelin and HDL-C (r= 0.11, P ≤ 0.05) in G2 and (r= 0.15, P ≥ 0.05) in G3, as revealed in Table (3-9).

### 3.9.9 Correlation of Serum Ghrelin and LDL-C:

There was a negative correlation between serum ghrelin and LDL-C (r= -0.19, P ≥ 0.05), (r= -0.19, P ≥ 0.05) in $G_2$ and $G_3$ respectively as revealed in Table (3-9).

### 3.9.10 Correlation of Serum Ghrelin and VLDL

There was a negative correlation between serum ghrelin and VLDL (r= 0.25, P ≥ 0.05) in G2 and (r= 0.10, P ≥ 0.05) in G3 as revealed in Table (3-9).

### 3.9.11 Correlation of Serum Ghrelin and Urea:

There was a negative correlation between serum ghrelin and urea (r= 0.24, P ≥ 0.05) in G2, (r= 0.14, P ≥ 0.05) in G3 respectively as revealed in Table (3-9).

### 3.9.12 Correlation of Serum Ghrelin and Creatinine:

There was a substantial negative correlation between serum ghrelin and creatinin (r= 0.48, P ≤ 0.01) in G2 and (r= 0.29 P ≤ 0.05) in G3 , as revealed in Table (3-9).

### 3.9.13 Correlation of Serum Ghrelin and Ferritin:

There was a negative correlation between serum ghrelin and ferritin (r= -0. 04, $P \geq 0.05$) in $G_2$ and (r= - 0.13, $P \geq 0.05$) in $G_3$, as revealed in Table (3-9).

### 3.9.14 Correlation of Serum Ghrelin and Cortisol

There was a positive correlation between serum ghrelin and cortisol (r= 0.07, $P \geq 0.05$) in $G_2$, while there was a negative correlation (r= -0.01, $P \geq 0.05$) in $G_3$ as revealed in Table (3-9).

### 3.9.15 Correlation of Serum Ghrelin and Testosterone:

There was a positive correlation between serum ghrelin and there was a significant positive correlation between serum ghrelin and testosterone (r= 0.15, $P \geq 0.05$) in G3, while was a substantial positive correlation between serum ghrelin and testosterone in $G_2$ respectively as shown a Table ( 3-9 ).

# *Conclusions*

1- Serum FSG, insulin, and HOMA-IR levels were significantly increased in MI patients (with / without DM) as paralleled to the controls, which means that all patients had hyperglycemia, hyperinsulinemia, and IR.

2- Serum ghrelin level was highly significant decrease in MI patients with DM as paralleled to MI patients and controls.

3- Serum TC and TG, LDL-C, and VLDL were significantly increased in MI patients (with / without DM) as paralleled to the control. While, there was a significant decrease in serum HDL-C in MI patients (with / without DM) as paralleled to the control.

4- Serum ferritin level was highly significant increased in MI patients with DM as paralleled to MI patients and controls.

5- Serum cortisol level was highly significant increased in MI patients (with / without DM) as paralleled to the controls.

6- Also, there was a reduction in serum testosterone levels in MI patients (with / without DM) as paralleled to the controls.

# *Suggestions for Future Studies*

1- Evaluation thepotential role for ghrelin based pharmacotherapies.

2- Study the role of serum ghrelin in diabetic patients with another complications such as nephropathy, or retinopathy.

3- Assessing the role of serum ghrelin in T1DM.

# (Appendix)

## Questionnaire:

Sample No:

Name:

Sex: age:

Smoking: Blood pressure:

Family history: medication:

Duration of DM:

History of MI or DM:

## Examination:

Weight: Height: BMI:

## Investigations:

Biochemical investigation

1) Ghrelin 2) Urea 3) Creatinine

4) Cortisol 5) Testosteron 6) Ferritin

7) FBS 8) Insulin 9) HOMA-IR

10) TC 11) TG 12) HDL

13) LDL 14) VLDL

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# الملخص


داء السكري هو مرض يتسم بأضطراب التمثيل الغذائي وارتفاع سكر الدم بشكل غير طبيعي نتيجة انخفاض مستوياته يتبعه انخفاض في مستويات الأنسولين مع أو بدون المقاومة غير المنتظمة لعمل الأنسولين . السكري هو عامل خطر رئيسي لمرض القلب والأوعية الدموية . الكثير من المرضى والوفيات تحدث نتيجة مضاعفات مرض السكر و تكون مسؤولة عن اضطرابات الدهون والتي هي احد الأسباب الرئيسية في أمراض القلب والأوعية الدموية .

الكريلين هو هرمون بيبتيدي مسؤول عن التغير في توازن الطاقة المتضرره من التغذية وافراز هرمون النمو علاوة على ذلك فهو يتحكم في أيض الكلوكوز و يسبب اضطرابات وتغيرات وظيفية متنوعة مثل امراض القلب والسكري.

شملت هذه الدراسة المرضى في مستشفى ابن النفيس التعليمي والمركز العراقي لامراض القلب / بغداد للفترة من ديسمبر 2018 الى فبراير 2019.

تم اختيار المرضى الذين يعانون من احتشاء عضلة القلب الحاد مع وبدون مرض السكري من النوع 2 . تحتوي الدراسة على100 مريض (50 يعانون من مرض السكري و50 بدون مرض سكري) الذي تتراوح اعمارهم بين 65-40 سنة . تم تشخيص المرضى الذين يعانون من احتشاء عضلة القلب على اساس التقارير الطبية و الفحوصات المختبرية و السريرية و اطباء استشاري أمراض القلب في المستشفيات المذكورة آنفا.

تمت مقارنة النتائج لمرضى أحتشاء عضلة القلب مع 50 سيط تتراوح أعمارهم

(65-35) سنة. تم أختيار مجموعة السيطرة على أنهم يبدون اصحاء ولايعانون من أمراض القلب ، السكري و أرتفاع ضغط الدم.

شملت الدراسة الحالية العديد من المؤشرات كالجلوكوز' صورة الدهون ' الانسولين في المصل ، الجريلين ، الفيريتين ' الكورتيزول ، التستوستيرون . وحددت العلاقة الأرتباطية بين مصل الجريلين والمؤشرات المذكوره أعلاه .

اظهرت النتائج زياده كبيرة في مؤشر كتلة الجسم ، الجلوكوز في الدم ، الكولسترول الكلي ، الدهون الثلاثية ، الكولسترول الدهني منخفض الكثافة ، الكرياتنين ، الفيريتين و الكورتيزول في مرضى احتشاء عضلة القلب الذين يعانون من مرض السكري مقارنه مع الذين لا يعانون من مرض السكري ، في حين كان هناك انخفاض كبير في مرضى احتشاء عضلة القلب الذين يعانون من مرض السكري مقارنه مع الذين لا يعانون من مرض السكري.

كان مرضى احتشاء عضلة القلب (مع / بدون مرض السكري) يعانون من خلل في الدهون ، مقاومة الانسولين ، مستويات منخفضة من هرمون الجريلين في الدم ، التستوستيرون ، خلل في اليوريا و الكرياتنين.

تهدف الدراسة الحالية على تقيم مستويات الكريلين في المصل لدى امراض داء السكري النوع الثاني والمصاحب لأمراض القلب والأوعية الدموية فضلا عن تقيم مستويات الفريتين والكورتيزول والتستوستيرون والأنسولين وصورة الدهون ومقارنتها مع الأصحاء.

**Republic of Iraq**

**Ministry of Higher Education and Scientific Research**

**University of Baghdad**

**College of Education for Pure Science (Ibn Al-Haitham)**

**Department of Chemistry**

# Evaluation of Serum Ghrelin and Some Biochemical Factors in Type2 Diabetic Iraqi Patients with Cardiovascular Disease

A thesis

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**By**

**Hamsa Ghawamer Ghazy**

B.Sc. Chemistry (2016)/ College of Education for pure Sciences/ Ibn Al-Haitham/ University of Baghdad

**Supervised By**

**Dr. Nawal. M. J. Al-Shammaa**

**Assistant Professor**

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