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Evaluation of Some Hormones, Lipid Profile and Oxidant/Antioxidant Concentration in The Type I Diabetes Mellitus Patients in Karbala Province

A Thesis

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Submitted by

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بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

﴿ قَالَ رَبِّ ٱشْرَحْ لِي صَدْرِي (٢٥) وَيَسِّرْ لِيٓ أَمْرِي (٢٦)
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Dedication

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Supervisor certification

We certify that this thesis entitles (**Evaluation of Some Hormones, Lipid Profile and Oxidant/Antioxidant Concentration in The Type I Diabetes Mellitus Patients in Karbala Province**) was prepared under my supervision at the department of Clinical Laboratories, at the College of Applied Medical Sciences, University of Kerbala, as a partial requirement for the degree of Master in Clinical Laboratories.

Signature

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Recommendation of the department chair

In view of the available recommendation, I forward this thesis for debate by the examining committee.

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Linguistic Certification

Certify that the master's thesis tagged: (Evaluation of Some Hormones, Lipid Profile and Oxidant/Antioxidant Concentration in The Type I Diabetes Mellitus Patients in Karbala Province) by the master's student (Rieam Dakhel Muhsen), in the Department of Clinical Laboratories - College of Applied Medical Sciences – Univ. of Kerbala, was reviewed from the linguistic point of view, and all the grammatical and stylistic errors in it were corrected, and the thesis is eligible for the discussion.

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We are member of the discussion committee certify that we read this thesis entitled " **Evaluation of Some Hormones, Lipid Profile and Oxidant/Antioxidant Concentration in The Type I Diabetes Mellitus Patients in Karbala Province**" and we discussed Mr. student **Rieam Dakhel Muhsen** in its contents. It is adequate for the award of the certification of Master of Clinical Laboratories.

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I have certified upon the discussion of the examining committee.

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Dean of the College of Applied Medical Sciences / University of Kerbala

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List of Abbreviations

Abbreviations	Items
AD	Alzheimer's disease
ADA	American Diabetes Association
ADP	Adenosine diphosphate
AG	Adjutant General
AIDS	Acquired Immune Deficiency Syndrome
ALS	Amyotrophic Lateral Sclerosis
ApoA-I	Apolipoprotein A-I
ApoA-II	Apolipoprotein A-II
Arg	Arginine
ASCVD	Atherosclerotic Cardiovascular Disease
ATP	Adenosine triphosphate
BAT	Brown adipose tissue
BBR	Basal Bolus Regimens
BMI	Body Mass Index
CAT	Catalase
CCK	Cholecystokinin
CHO	Cholesterol
CKD	Chronic Kidney Disease
CNS	Central Nervous System
CR	Conventional Regimen
CSII	Continuous Subcutaneous Insulin Infusion
Cu	Copper
CVD	Cardiovascular Disease
DFU	Diabetes foot ulcer
DKA	Diabetic Ketoacidosis
DKD	Diabetic kidney Disease
DM	Diabetes Mellitus
DN	Diabetic Nephropathy
DNA	Deoxyribonucleic Acid
DNe	Diabetic Neuropathy
DR	Diabetic Retinopathy
DSBmT	m-toluidine-disodium
EDTA	Ethylenediaminetetraacetic Acid
ELISA	Enzyme-Linked Immunosorbent Assay
ESRD	End-Stage Renal Disease
FFA	Free fatty acid
FPG	Fasting Plasma Glucose
G6PDH	Glucose-6-phosphate Dehydrogenase
GAD-65	65 kDa Glutamic Acid Decarboxylase
GBM	Glomerular Basement Membrane
GDM	Gestational Diabetes Mellitus
GFR	Glomerular Filtration Rate

GH	Growth Hormone
GHRL	Ghrelin
GHSR1a	Growth Hormone Secretagogue Receptor 1a
GI hormones	Gastrointestinal Hormones
GLUT4	Glucose Transporter Type 4
GSH	Glutathione
GSHGPx	Glutathione Peroxidase
GSSG	Glutathione Disulfide
HbA1c	Hemoglobin A1C
HDL	High-Density Lipoprotein
HDL-C	High-Density Lipoprotein-Cholesterol
HEPES Buffer	N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid
HIV	Human Immunodeficiency Virus
HK	Hexokinase
HLA	Human Leukocyte Antigens
IDDM	Insulin-Dependent Diabetes Mellitus
IDF	International Diabetes Federation
IFCC	International Federation of Clinical Chemistry
IR	Insulin Resistance
LDL	Low-Density Lipoprotein
LEP	Leptin
LEPR	Leptin acts by binding to its membrane Receptor
LPO	Lipid Peroxidation
Lys	Lysine
MDA	Malondialdehyde
MDI	Multiple Daily Injections
MES buffer	2-Morpholinoethanesulfonic Acid
Mg	Magnesium
MHC	Major Histocompatibility Complex
mRNA	Messenger Ribonucleic Acid
NAD	Nicotinamide Adenine Dinucleotide
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NIDDM	Non-Insulin-Dependent Diabetes Mellitus
NOS	Nitro-Oxidative Stress
O2	Oxygen
OB	Obestatin
OB-R	The Leptin Receptor
OD	Optical Density
OGTT	Oral Glucose Tolerance Test
OS	Oxidative Stress
Pro	Proline
RBC	Red Blood Cells
RBS	Random Blood Sugar
RCT	Randomized Controlled Trials

RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
Se	Selenium
SOD	Superoxide Dismutase
SPSS	Statistical package for the social sciences
T1D-GC	Type 1 Diabetes Genetics Consortium
T1DM	Type I Diabetes Mellitus
T2DM	Type II Diabetes Mellitus
TAG	Triacylglycerol's
TB	Tuberculosis
TC	Total Cholesterol
TG	Triacylglycerol's or Triglyceride
TNF-α	Tumor Necrosis Factor-α
TRL	Triglyceride-Rich Lipoproteins
TSH	Thyroid Stimulating Hormone
U.S.A	United States of America
UAG	Upper gastrointestinal
UGI	Upper Gastrointestinal
VLDL	Very Low-Density Lipoprotein
WHO's	World health organization
Zn	Zinc
ZNT8	Zinc Transporter

Summary

Diabetes mellitus a group of metabolic diseases characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both can be broadly categorized into type I diabetes mellitus (destruction of pancreatic beta cells, causing absolute deficiency of insulin), type II diabetes mellitus (a combination of decreased insulin secretion and decreased insulin sensitivity (i.e. insulin resistance), gestational diabetes mellitus , other specific types of diabetes mellitus.

A study has been conducted during 6 months between November/2021 and April/2022 in Al-Hassan centre for diabetes and endocrinology in Al-Husain medical city of Karbala. The total number of participants are (240) person include (150) patients with type I diabetes mellitus (TIDM), the males (54) (36 %), the females 96 (64%) and (90) healthy control group, males (36) (40%), females (54) (60%). 5 ml sample of venous blood was obtained from patients and healthy. In addition to, some information was taken from each person which includes (age, gender, duration history of disease, length and weight). ABO group, glucose and lipid profile was done by manual kit and auto chemistry analyzer, hemoglobin analyzer to measure HbA1c. Enzyme-linked immunosorbent assay (ELISA) tests to measure MDA and GSH and hormones (Leptin, Ghrelin, Obestatin and CCK). All the measurement was done in Al Naqaa privet laboratory, except for the HbA1c which were done in the Al- Hassan Center.

In our study observed, the highest rate of age group in patients with T1DM in (12-17) years (48%) and (18-23) years (16%) confirms that high percentage of patients are children and adolescents. Also the most patients recorded in this study is a females and their percentage was (64%) while the males was (36%) confirms the most patients were diagnosed with diabetes are female this result demonstrated

a relationship between gender and type I diabetes. also showed a correlation between weight, height and body mass index (BMI) with gender. The considerable percentage of blood group for patients was showed in O (48%) and A (25.43%). Furthermore, observed increase in level of HbA1c and glucose concentrations in diabetic patients especially in females than males compared to the control group.

Also observed significant increase in concentrations of serum (MDA) and significant decreased in (GSH) concentrations in patients with T1DM compared to control. There has been a significant increase in concentrations of each one of the hormones (Leptin, Ghrelin, Obestatin and CCK) in patient's females than males when compared with control. also observed a significant increase in the levels of total TC, TG, LDL and VLDL and a considerable decrease in levels of HDL in patients when compared with control, the rise in the lipid profile showed in females larger than males. This study clarified a several relationships between the physiological parameters in patients' group, there were significant positive correlation between age with duration, height, weight and BMI, also found positive correlation between TC with TG and LDL and positive correlation between TG and VLDL. In addition to, positive correlation between CCK with leptin, Ghrelin, Obestatin and MDA, also there has been a positive correlation between leptin and both Ghrelin, Obestatin and MDA.

Chapter One

Introduction

Chapter One

Introduction

One of the most prevalent endocrine disorders that are caused by deficiencies in insulin action or insulin secretion is DM (RezRezaeiaei *et al.*, 2018). The primary sign of the diabetes is hyperglycemia in the blood, caused by insufficient pancreatic insulin secretion or low insulin-directed fostering of the glucose via target cells, DM could be classified to a number of the types, T1DM and T2DM are the two most common types, For T1DM, insulin renewal therapy is the backbone, while in T2DM, there should be lifestyle modification and a control diet (Yaribeygi, Butler, *et al*., 2019). Insulin-dependent diabetes mellitus (IDDM, or type 1), which is identified by absolute lack of the circulating insulin, while the non-insulin-dependent DM (NIDDM, or type II), which is identified by the increased levels of insulin that are ineffective at bringing blood sugar levels back to normal or through the impaired secretion of the insulin (Kamil *et al*., 2010).

T1DM is defined by the American Diabetes Association (ADA) as an insulin autoimmune process that destroys beta cells, typically resulting in absolute insulin deficiency, and T2DM is defined as progressive loss of the β cell insulin secretion which often happens in a context of the insulin resistance (IR) (Eizirik *et al*., 2020). The immune system of the body attacks the pancreatic beta cells that produce insulin in T1DM, which is an autoimmune disease, without insulin, glucose can't be converted to energy, which leads to elevated levels of the blood glucose (Araia *et al.*, 2017).

T1DM is common worldwide and steadily increasing in frequency of incidence of about 3 % yearly, T1DM is responsible for about 5 % to 10% of total population of individuals who have diabetes, whereas T2DM has almost all cases,

the number of individuals affected is predicted to rise to 642 million in 2040 (D'souza et al., 2017). In contrast to T2DM, in which both IR and decreased insulin secretion via the cells play a synergistic role, the pathogenesis regarding T1DM is caused by environmental, genetic, and immunologic factors which destroy the beta cells of endocrine pancreas and result in the insulin deficiency, it typically progresses over some period of several months to years throughout which period patients are asymptomatic, glycemic, and positive for the relevant autoantibodies, the autoimmune destruction process occurs in the persons who are genetically susceptible under triggering effects of at least one environmental factor *(*Paschou *et al.,* 2018).

The findings show that T1DM is becoming more common and prevalent worldwide, thus, insulin will be expensive and difficult to obtain, particularly in poor and undeveloped nations (Mobasseri *et al*., 2020). Low levels of glycemic control have lately been found in Iraq (10 % and 23 %), the low reported numbers were ascribed to social unrest, which also affected the provision of healthcare services and had an impact on pharmaceutical storage, difficulty with transporting insulin, and availability (Hadi, Al-Kaseer and Al-Zubaidi *et al.*, 2018).

Poor glycemic control in T1DM typically results in increased oxidative stress, diabetic complications, and oxygen-free radical generation (Alghobashy *et al*., 2018). The Reactive Oxygen and Nitrogen Species (ROS and RNS) are formed in higher fluxes under pathological conditions. They cause cellular damage due to the oxidation of amino acid residues on proteins, forming protein carbonyls (Kennedy *et al.*, 2020).

A significant upstream event for increasing free radical generation is diabetes, uncontrolled diabetes could cause the generation of free radicals (Ganjifrockwala *et al*., 2017). By interfering with the state of lipids, proteins, and DNA, oxidative

stress, which is imbalance between antioxidants and pro-oxidants in cells, might cause cellular damage (Zhang *et al.*, 2017). Active biomolecules known as free radicals are produced physiologically throughout metabolic pathways and/or by the immune cells, free radicals play physiological functions in a wide range of molecular processes, such as synaptic plasticity, cellular signaling, defense against pathogen invasion, memory formation, cell proliferation, cell-cell interactions, apoptosis, autophagy, and aging (van der Schaft *et al.*, 2019). oxidative stress is caused when the production of free cells exceeds the physiological range and overcome the antioxidant defenses of the cell, this damages DNA and proteins (Yaribeygi *et al*., 2020). Many different enzymes, which include catalase (CAT), superoxide dismutase (SOD), and glutathione (GSH), are part of the intrinsic defensive system that most biological cells have to defend themselves from attacks by free radical (Sifuentes-Franco *et al*., 2017). Because they have at least one electron in the outer layer of their orbitals, free radical species can interact directly with biological molecules like amino acids, lipids, and nucleic acids to attack cellular structures, many types of biological and chemical damage, including apoptosis, necrosis, lipid peroxidation, defects in the intra-cellular signalling pathways, protein breakages, DNA damage, and cell death, could happen based on the quantity of the overload of the free radicals (Yaribeygi, Butler, *et al.*, 2019).

Antioxidants are substances which stop other compounds from oxidizing or neutralize free radicals (Yashin *et al.*, 2017). This defense mechanism is disrupted in diseases like diabetes and other clinical states, and insufficient scavenging of RNS and ROS significantly contributes to tissue damage in the diabetic patients, the patient's quality of life and lifespan are negatively impacted by the increased abundance of free radicals because they activate stress-signaling pathways and

deplete both nonenzymatic and enzymatic antioxidants (Ganjifrockwala *et al.*, 2017).

Aim of the Study:

1- Evaluation the concentration of the oxidant/antioxidant (GSH, MDA) and relationship in patient with T1DM
2- Evaluation the concentration of the hormones (leptin, Ghrelin, Obestatim and Cholecytokinine) and relationship in patient with T1DM.
3- Measured the lipid profile parameters to see changes in patients with T1DM
4- Measured RBS and HbA1c and relationship in patient with T1DM
5- Identify the role of age, gender, weight and BMI and relationship in patient with T1DM.

Chapter two

Literatures Review

Chapter two

Literatures Review

2. Diabetes Mellitus

Increased blood glucose levels (i.e. hyperglycemia) characterized by deficiencies in the insulin actions, insulin secretion, or both are a hallmark of the metabolic disease DM, the pancreas's beta cells produce the hormone insulin, which the body needs to use the glucose from digested meals as an energy source (Ubeid *et al*., 2020). chronic hyperglycemia is linked to macrovascular and microvascular complications which might result in renal failure, amputations, nerve damage, blindness, and visual impairment (Ubeid *et al*., 2020). In addition, DM is defined as a chronic condition characterized by hyperglycemia brought on by impaired insulin action, insulin secretion, or both, it is well established that low-grade chronic inflammations and oxidative stresses contribute to the onset of DM and its consequences (Landon *et al*., 2020).

2.1. Types of Diabetes Mellitus

2.1.1. Type I Diabetes Mellitus

After asthma and epilepsy, T1DM is the 3rd most prevalent chronic disorder in children (Alabedi *et al*., 2020). T1DM represents a chronic autoimmune disease that is marked by an elevation in the blood glucose levels (hyperglycemia), characterized by an insulin deficiency that results from the loss or apoptosis of pancreatic islet β-cell, necessitating the use of exogenous insulin therapy for the rest of one's life (Katsarou *et al.*, 2017). T1DM, also known as insulin-dependent diabetes(IDDM), represents most frequent in young people and children but can happen at any age, T1DM patients cannot make enough insulin. Approximately 5 to 10 % of all diabetes cases are of this type, in this type, the pancreas is where beta cells are destroyed on

a cellular level, the pancreas does not release any insulin when a person has T1DM (Mobasseri *et al*., 2020).

The intricate interaction between resident or invading macrophages and T cells that release cytokines and chemokines in islet micro-environment and deliver cell-cell pro-apoptotic signals, and beta-cells through physiological signals or by stressed, injured, or dying beta-cells which activate and attract immune cells to islets, such interaction is influenced by host's genetic background, age, and environmental factors like diet and viral infections are shown in Fig 2.1 *(*Eizirik *et al.*, 2020). the first β¬cell-targeting autoantibody to manifest throughout the early years of childhood typically targets the insulin or GAD-65, the sort of autoantibody which arises first relies on environmental trigger as well as the genetic variables (Jónsdóttir *et al*., 2017).

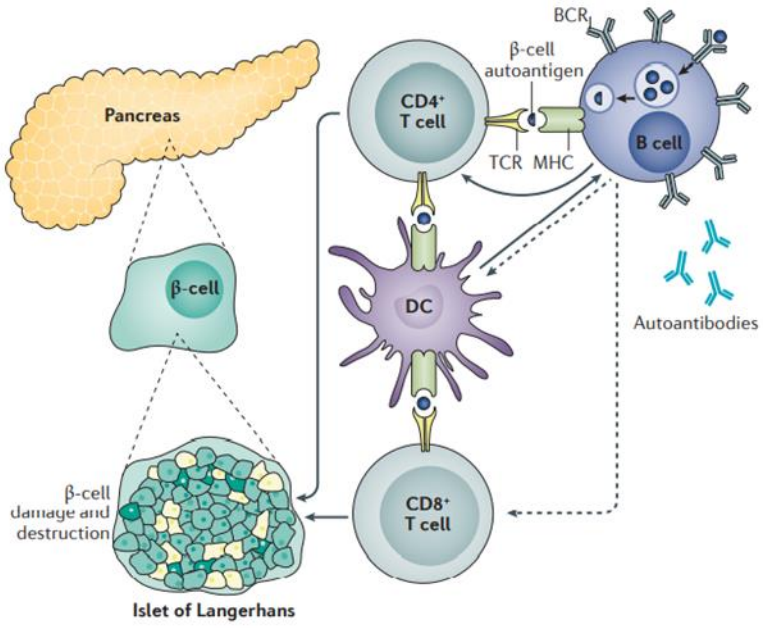


Figure 2.1 | Pathogenesis of T1DM (Katsarou et al., 2017)**.**

patients are dependent on the life-time injections of the insulin due to the fact that there isn't any cure; nevertheless, the innovative approaches for the treatment of the diabetes with the insulin, like continuous monitoring of the glucose, insulin pumps, and hybrid closed-loop systems, are now under development, even though the prevalence of macro vascular and microvascular problems had been reduced as a result of the strict glycemic control (Kastarou *etal*., 2017).

2.1.2. Type II Diabetes Mellitus

Is characterized by both IR and insufficient insulin secretion to meet the needs of the body (Gajewska *et al*., 2020). T2DM is a metabolic condition that is characterized through a decrease in the insulin secretion and peripheral IR(Guerra & Gastaldelli *et al*., 2020). there have been more than twice as many patients diagnosed with T2DM globally between 1980 and 2008, studies on the incidence of diabetes in persons aged 20 to 79 predicted that 285 million adults had T2DM in the year 2010, with a prevalence of 6.4 % globally, T2DM is expected to affect 439 million adults by the year 2030, or 7.7 % of the adult population globally (Witka *et al*., 2019). 422 million people all over the world have diabetes, according to the WHO's most recent figures, almost all of such T2DMs, where the body still generates insulin but is unable to properly use it (Walton *et al*., 2017). Patients with T2DM are more susceptible to many long- and short-term consequences, such as macrovascular disease and micro-vascular diseases, these patients might require insulin injections if the condition could not be managed with an oral medication (Chaudhury *et al*., 2017). Additionally, the condition has a strong genetic basis, and diabetes with a first-degree family history are more likely to develop the condition, this risk is multiplied by two if the two parents have the condition (Witka *et al.*, 2019).

Through the stimulation of the glucose uptake into adipose tissue and skeletal muscle, insulin reduces blood glucose in part (Chadt & Al-Hasani *et al*., 2020).

Pancreatic beta cells release insulin into blood-stream in the case where blood sugar levels rise shortly after eating, in a healthy expression, insulin stimulates peripheral tissues to express more transporters of the glucose, especially GLUT-4, at plasma membrane, opening cellular flood gates to the glucose, however, GLUT4 expression is actually lowered in chronically elevated insulin levels (Fazakerley *et al*., 2018).

Foods with lower energy densities are generally thought to be more protective against T2DM compared to foods with higher densities, and sugar-sweetened beverages and refined grains consistently seem to increase risks of diabetes and obesity (Jannasch *et al.*, 2017).

2.1.3. Gestational Diabetes Mellitus (GDM)

Conventional definitions of GDM include carbohydrate intolerance of varying severity that begins or is first detected throughout pregnancy, GDM is extremely harmful to both the mother and the fetus (Gao *et al*., 2019). GDM prevalence among pregnant women is between 1-14 % globally, one in every four pregnancies, 90 % of which are GDM, is affected by hyperglycemia *(*Balaji *et al.*, 2019). It is well known and widely acknowledged that women who are diagnosed with GDM have a greater chance of developing T2DM in the future (Szmuilowicz *et al*., 2019). women and their babies are more likely to have long-term health issues like T1DM and disabilities (Han *et al.*, 2017).

Risk factors include obesity/overweight, micronutrient deficiencies and westernized diet, a family history of diabetes/IR, and advanced maternal age (Plows *et al*., 2018). Typically, oral glucose tolerance test (OGTT) is used for diagnosis (McIntyre *et al.*, 2019). early prenatal screening during the first trimester or at the start of antenatal treatment is often advised, an OGTT is used to screen for GDM later in pregnancy between 24 and 28 weeks of gestation (Johns *et al.*, 2018). The

main treatments for GDM are increased physical activity and dietary changes, however medication, mostly insulin, is utilized when normoglycemia is not attained (McIntyre *et al.,* 2019).

2.1.4. Other type

The other DM types include maturity-onset diabetes in young, latent autoimmune diabetes in the adult patients, a genetic form of diabetes, and secondary diabetes brought on by other conditions like pancreatitis or as a side effect of medications like corticosteroids (Yaribeygi et al., 2020). Types of diabetes brought on by other factors include, for example, monogenic syndromes of diabetes (like maturity-onset diabetes and neonatal diabetes in the young), exocrine pancreatic diseases (like pancreatitis and cystic fibrosis), and chemical- or drug-induced diabetes (like using glucocorticoids, in treatments of HIV/AIDS, or after transplantation of an organ) (Care & Suppl *et al*., 2021).

2.1.1.1. Classification of T1DM

The prevalence of non-autoimmune varieties of T1DM linked to insulin insufficiency, like fulminant T1DM and virus-induced T1DM, as well as other different types of atypical diabetes, varies among Asian nations along with the conventional autoimmune T1DM, particularly in older children and adults *(*Lee *et al*., 2019).

2.1.1.2. Epidemiology of T1DM

The great majority of instances of diabetes are caused by T2DM, whereas T1DM diabetes affects between 5 and 10 % of all people with diabetes (*Gajewska et al.,* 2020). With a prevalence of 1 in every 300 people and a yearly increase in frequency of roughly 3%, T1DM is relatively common in the world (Robert *et al*., 2018).

The immunologic characteristics and epidemiology of T1DM differ significantly between Caucasians and Asians, the East Asian nations of China, Korea, and Japan have the lowest reported rates of T1DM worldwide (Lee *et al*., 2019). Yet, several nations, including Sardinia, Kuwait, and Puerto Rico, exhibit an unexplained sharp increase in the incidence rate (Robert *et al.*, 2018). The environment and genetic background of island's residents make it an optimal area for researching immunological, environmental, and genetic factors that are associated with the etio-pathogenesis of T1DM, The island of Sardinia has the 2nd highest T1DM incidence worldwide (45/100,000), right after Finland (64.2/100,000) (Songini *et al*., 2017).

because there are some regional and national diabetes registries to support research, offer trustworthy data, and assist in addressing the disease's pervasive threat, an Arab population-based diabetes registry is urgently needed, where There are few statistics from Iraq (Almahfoodh *et al.*, 2017). The prevalence of T1DM increased between 2012 and 2016 in Basra City, comparable to other cities, also Al-Nassiryah City's incidence of diabetes had been rising over the last five years (Zalzala *et al.,* 2020).

2.1.1.3. Risk factor of T1DM

Even though etiology of T1DM is not fully known, T1DM is the result of a combination of many environmental and genetic variables can contribute to T1DM's development (Norris *et al*., 2020).

T1DM has a complicated genetic background; while some genes have been identified as protective, others have been identified as causative, for instance, the main gene associated with the majority of T1DM patients is IDDM1, which is found on human leukocyte antigen (HLA) II on the chromosome 6, yet, since not all T1DM

cases have the same genetic mutations, it is challenging to explain the genetic background regarding T1DM depending on HLA involvement alone(Alhazmi *et al*., 2020).

Environmental triggers that affect the etiology of T1DM include viral infections and dietary agents which they may trigger autoimmune or hasten the loss of beta cells that eventually results in T1DM (Songini *et al*., 2017).

2.1.1.4. Symptoms of T1DM

The majority of the time, symptoms begin in adolescence or childhood, while they can often appear much later in life (Robert *et al.*, 2018). Even though the severity of the symptoms varies, all types of diabetes share the same symptoms, T1DM symptoms appeared more quickly compared to T2DM (Care & Suppl *et al*., 2021). The most typical diabetes signs include: excessive thirst, frequent urination, increased tiredness, weight gain, intense hunger, blurred vision, irritability, itchy skin, skin and/or yeast infections, frequent gum disease/infection, red and/or swollen gums that pull away from teeth, tingling or numbness, particularly in the hands and feet, as well as male sexual dysfunction (Begic *et al*., 2016). upper gastrointestinal (UGI) symptoms are rather prevalent, vomiting, nausea, early satiety, postprandial fullness, belching, bloating, heartburn, abdominal pains, and weight loss were among the symptoms that were reported, diabetes is linked to gastrointestinal dysmotility, which can cause symptoms similar to functional dyspepsia and gastroparesis, delayed gastric emptying (Chedid *et al*., 2019). leading to protein and lipid metabolic disorders: 1 The long-term hypoglycemia effects are organ and tissue damages, 2 Symptoms of diabetes include thirst, polyuria, weight loss, and disorders in the vision (Norris *et al*., 2020).

One of the contributing factors behind children's severe growth retardation was T1DM (Hadi *et al*., 2018). Hyperosmolar and more severe forms of diabetic ketoacidosis can occasionally cause coma and stupor, yet the majority of symptoms are severe, which over time may harm or even result in the failure of several organs, leading to permanent harm like amputation, blindness, stroke, and ultimately death (Norris *et al.,* 2020).

2.1.1.5. Treatment of T1DM

in order to maintain appropriate blood glucose levels during fasting and postprandial periods, theoretically, such points can be attained by controlling one's diet, engaging in regular exercise, losing weight, taking an oral medication (only for T1DM) and receiving insulin injections (for T1DM and T2DM that has not responded to oral therapies, typically in long-term diabetics) (Sanlier & Gencer *et al*., 2020). The majority of T1DM patients depend on a lifetime of insulin injection therapy because the causes and risk factors connected with the disease are still poorly understood, making it impossible to create effective treatments or preventative measures, lately, innovative methods for treating insulin-related conditions were developed, including the use of continuous monitoring of the glucose, insulin pumps, and hybrid closed-loop systems (Robert *et al.,* 2018).

The discovery of the insulin in year 1922 led to a significant advancement in the therapy of diabetes, conventional and basal bolus insulin regimens are the two commonly used insulin regimens, although the traditional insulin regimen is still applied in certain places (Brink *et al*., 2022). The conventional regimen CR and the basal bolus regimens BBR (intensive therapy), which comprise multiple daily injections MDI and continuous subcutaneous insulin infusion CSII, MDI: increase physiologic insulin effects, more flexible, and decrease the risk of diabetic ketoacidosis, at least four daily injections, including during school hours, a constant

rather than adjustable basal rate (Alabedi *et al*., 2020). Despite the foregoing, it is important to keep in mind that there is no one ideal insulin regimen or meal plan, instead, the general rule is that diabetic care must blend as seamlessly as possible with the surrounding home, family's eating habits and preferences (Iqbal *et al.*, 2018).

Although the standard insulin regimen is still utilized in certain places, it is not the ideal regimen since it is a strict regimen that calls for the timing of injections, a specific meal size, a specific mealtime, and snacks to prevent hypoglycemia (Alabedi *et al*., 2020).

2.1.1.6. Complications of Diabetes Mellitus

Chronic metabolic disease DM could cause numerous long-term macrovascular and microvascular problems are shown in Fig 2.2 (Yamazaki *et al.*, 2018).

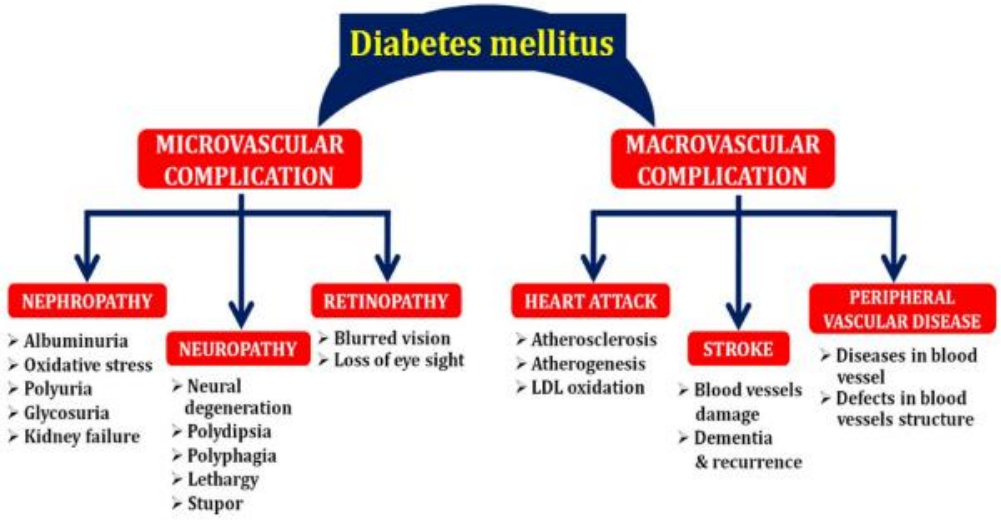


Fig. 2.2 Diabetes mediated macro- and micro- vascular complications (Naveen & Baskaran, 2018)

Micro and Macro Vascular Complication

When DM affects the large blood vessels in the brain and heart, it can result in vascular problems such hypertension, heart disease, and stroke (Papatheodorou *et al*., 2018). Vascular complications when DM impacts small blood vessels, such as diabetic nephropathy, diabetic retinopathy, and diabetic neuropathy, which represent the leading mortality and morbidity causes in the diabetic people around the world and current treatments are still ineffective (S. C. Kim *et al*., 2020). Blindness, End-stage renal disease (ESRD), and limb amputation are all well-known outcomes of diabetes (Di Vincenzo et al., 2019). Retinopathy, which could result in nephropathy, vision loss, which can cause peripheral neuropathy, renal failure, which can result in amputations, foot ulcers, Charcot joints, and autonomic neuropathy, which can result in genitourinary, gastrointestinal, and cardiovascular symptoms and sexual dysfunction, are a few of the long-term complications regarding diabetes (Goeijenbier *et al.,* 2017).

2.1.1.6.1. Diabetic Peripheral Neuropathy

Diabetes neuropathy is a very common illness that has a significant impact on patients by raising the risk of falls, resulting in discomfort, and lowering quality of life (Pop-Busui *et al*., 2017). There are likely a number of secondary causes for the increased incidence of neuropathy in people with T1DM or T2DM, including variations in the age at which diabetes first manifested itself and variations in the underlying pathophysiology (Feldman *et al.*, 2019).

Tingling, numbness, weakness, discomfort, and unsteadiness represent diabetic neuropathy signs. Those symptoms start distally (at toes), progress proximally, and after that spread to upper limb digits in a case when the symptoms of the lower-limb reach the knees (Feldman *et al*., 2019). Due to the peripheral

arterial disease or peripheral neuropathy, poor glycemic control, and foot deformity in DM patients, diabetic foot ulcers are wounds to all layers of skin that typically occur on the feet's soles, they can include infections, ulcerations, and gangrene (Monteiro-Soares *et al.,* 2020). Poor wound healing is the main morbidity and mortality cause in the diabetic patients, and up to 25% of diabetic people are under the risk of developing DFU during the course of their lives, important contributing factors to the development of DFU include hyperinsulinemia, hyperglycemia, dyslipidemia, increased bio-markers of inflammation, and oxidative stress (Mohseni *et al*., 2018). Although an individual with diabetes may experience a variety of complications, none are more severe compared to those involving the foot, DFU account for nearly 20% of hospitalizations for diabetes-related reasons (Ahmadishooli *et al.*, 2020).

2.1.1.6.2. Diabetic Retinopathy (DR)

The most prevalent and underestimated diabetic consequence is diabetic retinopathy (DR), the retinal microvasculature is impacted, and it's a sight-threatening, long-term side effect of diabetes that's connected to persistent hyperglycemia (W. Wang & Lo *et al*., 2018). The dilation of blood vessels and changes to blood flow are retinal blood vessels' first reactions to hyperglycemia, those changes have been considered to represent a metabolic auto-regulation that helps diabetic people' retinal metabolism (Bek *et al*., 2017).

2.1.1.6.3. Diabetic Nephropathy (DN)

In developed countries, DN is the most frequent reason for end-stage renal disease (Zürbig *et al.,* 2019). In both T1DM and T2DM, DN, now more frequently referred to as diabetic kidney disease (DKD), keeps being a major source of mortality and morbidity, chronically, it has an impact on kidney health (Stroescu *et*

al., 2018). Thus, it is the main contributor to ESRD and chronic kidney disease globally and p robably also in high-income nations (G. Wang *et al.*, 2019). DN currently accounts for more than 50% of patients who need dialysis and/or a transplantation in various regions of the world, making it the most common cause of end-stage renal disease (Warren *et al*., 2019). By the year of 2040, CKD, which is mostly caused by DN, is predicted to rank as the 5th leading mortality cause worldwide and as the 2nd leading cause in long-lived nations (Ortiz *et al.*, 2019). Clinically, DN is typically defined by the occurrence of proteinuria or declining renal function, such as reduced glomerular filtration rate (GFR) (Donate-Correa *et al*., 2020). pathologically, DN is frequently identified by the glomerular basement membrane (GBM) thickening, glomerular mesangial matrix expansion, and glomerular nodular sclerosis formation in the advanced stages (Alicic *et al*., 2017). Patients with DN who have little to no albuminuria run the risk to develop ESRD (G. Wang *et al.*, 2019).

2.2. Oxidative Stress

Different metabolic activities naturally produce ROS, which are produced in small amounts during regular metabolism, ROS are highly reactive, as their name suggests, and remove electrons from anything that gets in their way, ROS harms cells when they are present in excess of what is normally physiological (Walton *et al*., 2017). Hydroxyl, superoxide, hydroperoxyl, and peroxyl radicals are examples of ROS, Hydrogen peroxide and hypochlorous acid are non-radical ROS examples, nitrogen dioxide, nitric oxide, nitrous oxide, and peroxynitrite are a few examples of RNS, which are created in small levels under the normal physiological conditions (Ganjifrockwala *et al*., 2017).

Considered to be a significant underlying factor in various clinical diseases, oxidative stress (Sifuentes-Franco *et al.*, 2017). An imbalance between antioxidants

and pro-oxidants in cells is referred to as oxidative stress, by interfering with the state of proteins, DNA, and lipids, oxidative stress might cause cellular damage and was related to development of numerous diseases (Yaribeygi, Atkin, *et al.*, 2019). Persistent hyperglycemia increases the generation of cytosolic and mitochondrial ROS and favors the dysregulation of antioxidant defenses, which could activate a state of metabolic pathways and cause endoplasmic reticulum stress and nitro-oxidative stress (NOS) (Sifuentes-Franco *et al*., 2017). A hyperglycemic environment is linked to oxidative stress, ROS prevent insulin-stimulated glucose uptake, ROS also impede the production of glycogen in the muscle and liver are shown in Fig 2.3 (Plows *et al*., 2018).

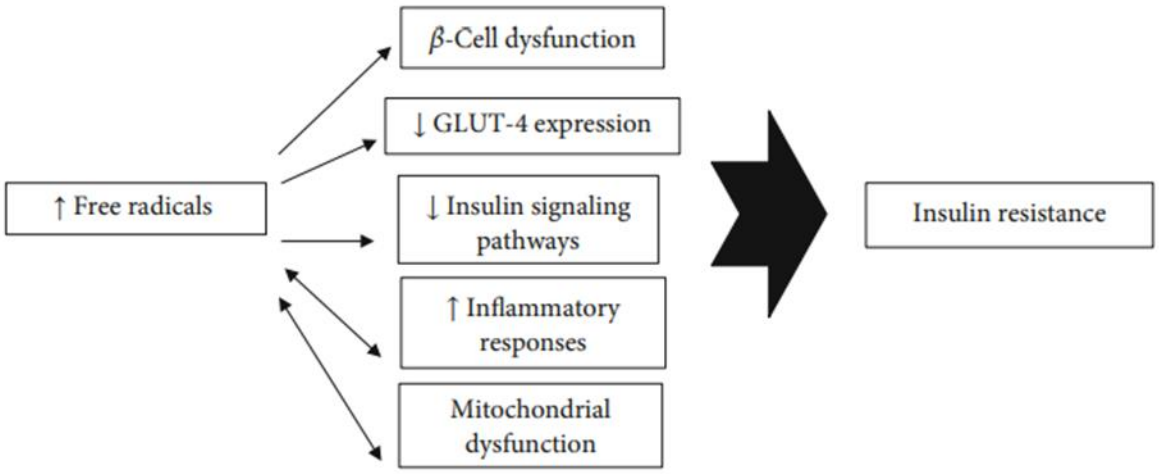


Figure2-3: Oxidative stress induces the insulin resistance through 5 major molecular path-ways (Yaribeygi et al., 2020)

Antioxidants are substances that stop other compounds from oxidizing or neutralize free radicals, the antioxidant system consists of molecules including uric acid, GSH, transferrin, albumin, vitamin C, lipoic acid, bilirubin, vitamin E, copper, carotenoid, and zinc as well as enzymes like catalase, SOD, and glutathione peroxidase (Ganjifrockwala *et al.*, 2017). the antioxidants scavenge free radicals by

certain mechanisms, free radicals are degraded by enzymes, proteins like transferrin could bind to metals that stimulate the formation of free radicals, and vitamins E and C serve as free radical scavengers, while vitamin E, a lipid-soluble vitamin, disrupts the chain events of lipid peroxidation, vitamin C, a water-soluble molecule, often scavenges hydroxyl radicals (Ganjifrockwala *et al.*, 2017).

2.3. Oxidant/Antioxidant

2.3.1. Glutathione (GSH)

Glutathione is a tripeptide consisting of glycine, cysteine and glutamic acid and is the most abundant non-protein thiol (Ahmadi *et al.*, 2017). occupies approximately 95% of non-protein thiol groups in vivo and is ubiquitously present in mammalian cells at concentrations of 0.5 to 10 mM, depending on the tissues (Fraternale *et al*., 2017). The degree of individual variability in glutathione production, mostly brought on by genetic variability in the enzymes involved in its regeneration and/or production, is a factor determining glutathione status (Minich & Brown *et al*., 2019). Glutathione, which is involved in the transport of amino acids, acts as a coenzyme for enzymes (Karolczak *et al.*, 2017). it plays a pivotal role in critical physiological processes resulting in effects relevant to diverse disease pathophysiology such as maintenance of redox balance, reduction of oxidative stress, enhancement of metabolic detoxification, and regulation of immune system function (Calabrese *et al*., 2017). Glutathione is regarded as the most significant anti-oxidant preventing the oxidative damage that free radicals cause to the cell membrane, it can exist in two states: a thiol-reduced state (GSH) and an oxidized state (GSSG), which is made up of 2 GSH molecules that are joined by a disulfide bond (Aoyama *et al*., 2021).

2.3.2. Malondialdehyde (MDA)

Malondialdehyde (MDA) is a small, reactive organic molecule that is present in all eukaryotes and is made up of 3 carbon molecules with 2 aldehyde groups at the carbon 3 and carbon 1 positions (Morales & Munné-Bosch *et al*., 2019). MDA is a specific aldehyde that was utilized as a bio-marker of oxidative stress and is thought to be an abundant product of lipid peroxidation (L. Wang *et al*., 2017). Malonaldehyde is a stable end product of lipid peroxidation which is a chain reaction providing a continuous supply of free radicals that initiate further peroxidation (Cui *et al*., 2018). The lipid peroxidation one of the major sources of ROS, therefore , MDA plasma levels reflect the magnitude of lipid peroxidation, but also the severity of oxidative stress (Maurya *et al*., 2021). Oxidative stress induces an overproduction of reactive nitrogen species (RNS) or reactive oxygen species (ROS), which can react with other biomolecules, such as lipids, to generate different compounds including Malondialdehyde (MDA), This phenomenon is known as lipid peroxidation (Isola *et al*., 2019).

2.4. Hormones

2.4.1. Insulin

A small protein called human insulin has 51 amino acids and is made up of two chains: An A-chain, which contains 21 amino acids, and the B-chain, which has 30 amino acids, those chains are joined by disulfide bonds (Shen *et al*., 2019). In insulin-producing pancreatic beta-cells on chromosome 11, humans have one insulin gene called INS (whereas rodents have 2, ins2 and ins1) (Piccinini & Bergman *et al*., 2020). And It is considered an important endocrine polypeptide hormone synthesized by the β-cells of the pancreas, that regulates carbohydrate metabolism, Where The actions of the hormone are initiated by binding to its receptor on the

surface of target cells, Where Abnormal levels of insulin are associated with diabetes mellitus that is characterized by chronic hyperglycemia (Petersen & Shulman *et al*., 2018).

The main tissues targeted by the insulin's effects on the metabolism include (for example, skeletal muscles, liver, and adipose tissue) , where the insulin promotes the uptake of the fatty acids and glucose and inhibits the lipolysis (Haeusler *et al.*, 2018).

Maintaining an appropriate blood glucose level is insulin's principal goal, By attaching to certain receptors that are found on liver, fat and muscle cells, to increase glucose uptake in this tissue which insulin-sensitive (Tokarz *et al*., 2018). After that, insulin stimulates glycogenesis in the liver, lastly, insulin signals the liver to stop generating glucose through gluconeogenesis and glycogenolysis, which inhibits glucagon secretion simultaneously, glycogen is converted back into glucose and released into blood if there is a low glucose level discovered in the blood (Mathieu *et al.*, 2017).

The liver serves as an insulin gateway, allowing only the right insulin mass to the organisms is proportionate to the metabolic needs (Maciejczyk *et al.*, 2019). Insulin is delivered to liver from pancreas in discrete pulses that happen every 5 minutes, the concentration of insulin that arrives at the liver via portal vein could be up to 10-times higher compared to concentration in peripheral circulation (Hatting *et al*., 2018). insulin that makes it through the liver's initial filtering stage travels to the systemic circulation through the hepatic veins, where it might operate on tissues, finally, it is eliminated by tissues that are sensitive to insulin, such as the kidneys, skeletal muscle, and the liver (after recirculation) (Saltiel *et al*., 2021). no longer detectable in circulation 30min after it is released from pancreas, and its half-life once in circulation is approximately 6min (Chan *et al.*, 2017).

2.4.2. Leptin

Leptin, which is expressed in various tissues, was discovered in 1994, the anorexigenic (appetite-suppressing) peptide hormone leptin, as well as its receptor, are widely distributed in a variety of tissues, it controls both energy expenditure and food intake(Onyemelukwe *et al*., 2020). leptin, a circulating adipokine, functions by attaching to its membrane receptor (LEPR, which is referred to as the OB-R as well), a single membrane-spanning receptor which has been discovered (Schuit *et al*., 2021). Leptin, the 167 amino acid product of the Lep (formerly ob.) gene, is one of the most potent signals, The levels of serum leptin are favorably correlated with the BMI, percentage of body fat, size of adipocytes, and fat mass, so recognized biomarker of adiposity(Pérez-Pérez *et al*., 2017). secreted into the bloodstream by white adipose tissue, additionally, leptin is expressed in the following tissues: mammary gland, brown adipose tissue (BAT), ovary, placenta, stomach, skeletal muscle, lymphoid tissue, and pituitary gland, yet, the relative contribution from such tissues to total circulating leptin levels is insignificant (D'souza *et al*., 2017). released from the small vesicles within adipocytes on a diurnal pulsatile basis, with higher rates early morning and in the evening, leptin is circulated in the serum after being secreted in both a bound form and free form (Schuit *et al*., 2021) .

leptin secretion is accelerated by the growth of adipocytes associated with obesity in humans, leading to higher serum leptin levels, this effect might also be brought on by chronic hyperinsulinemia and enhanced cortisol turnover, similar to this, eating less leads to weight loss, which lowers leptin levels, additionally, so serum leptin decrease throughout fasting and increase throughout the refeeding, exhibiting a pattern that is comparable to the release of insulin by pancreatic islets (Marques-Oliveira *et al*., 2018). The expression of leptin's mRNA and protein as well as its release by adipocytes appear to be increased by insulin (Marques-Oliveira

et al., 2018). Insulin and leptin functions are linked and help to maintain proper metabolic regulation, leptin decreases continuous calorie intake while insulin ensures proper energy use and storage (Poetsch *et al.*, 2020) .

Even when there is an abundance of leptin, leptin resistance occurs when hypothalamic neurons become of a lower or no sensitivity to the leptin, the primary causes of leptin resistance are mutations in leptin and LEPR genes, decreased plasma membrane expression of LEPR, worsening of LEPR functions and signaling, or changes in leptin transport across blood-brain barrier (Poetsch *et al*., 2020). Increased levels of leptin make patients more susceptible to diet-induced obesity, creating a vicious circle that raises leptin levels even more and worsens already-existing leptin resistance, showing that the actual leptin is a key factor in the emergence of "leptin-induced leptin resistance," which is when resistance to leptin develops due its own actions (Ramos-Lobo & Donato *et al*., 2017).

2.4.3. Ghrelin (GHRL)

The ghrelin gene (GHRL) produces the 28-amino acid peptide ghrelin, which is mostly released by oxyntic glands regarding the mucosa of the gastric fundus, The sole known receptor for endogenous acyl ghrelin is the GHSR-1a, which is expressed in the pancreatic islet from early gestation to adulthood, ghrelin is famously referred to as "hunger hormone." Ghrelin is understood to be the only circulating hormone peripherally stimulating food intake in both humans and animals (Napolitano *et al*., 2018). ghrelin exerts feeding patterns in the central nervous system (CNS) despite being released in upper gastrointestinal tract with the ability to control neuronal circuits which control energy expenditure and food intake (Gray *et al.*, 2019).

while the pancreas and other tissues also express ghrelin, the stomach is where it is largely produced, first revealed pancreatic ghrelin synthesis in humans in 2002, they identified a distinct population of islet cells that expressed ghrelin, these cells were initially referred to as "ghrelin" cells and were later given the expression "ε-cells." the primary source of ghrelin expression throughout the perinatal period is the pancreas (Deschaine *et al.*, 2022).

Insulin, leptin, and ghrelin are all known as regulators of appetite and energy homeostasis, ghrelin levels rise before meals and fall after the meals, insulin and ghrelin both show a reciprocal link throughout the day (Poher *et al.*, 2018). the levels of the insulin being high in the case where the levels of the ghrelin are low and vice versa, it is the antagonist regarding the hormone leptin, which is produced through adipose tissue and promotes a sense of satiety when it is present in high quantities (Warchoł *et al*., 2018).

2.4.4. Obestatin

Obestatin is a post-translationally modified 23-amino acid peptide hormone that has a C terminal amide group on Leu-23, the peptide is produced from a prepropeptide that shares 117 residues with ghrelin, the inclusion of an amide group at the C-terminus is assumed to have functional importance, as it does with various regulatory peptides(Freitas *et al.*, 2021). in the instance of obestatin, it was previously postulated that C-terminus was necessary for receptor binding, but it attracted attention for its function in regulating food intake, stomach emptying, and the reversal of high body weight, even though both are generated from the same precursor protein, it has biological actions that are in opposition to those of ghrelin (Green & Grieve *et al*., 2018).

Obestatin is mostly produced physiologically in the gastrointestinal, primarily in the stomach, although it is also produced in the duodenum, the ileum, and the jejunum, the colon is noticeably absent from its production, obestatin is present specifically in crypts regarding Lieberkuhn and Brunner's glands in the human intestine(Guo & Chen *et al*., 2021). although it can also be synthesized in the mammary gland, brain, and testicular cells, however, intriguingly, it has been found as well on periphery of exocrine pancreatic ducts and pancreatic islet (Irez *et al*., 2019). Obestatin slowed jejunal motility and gastric emptying, reduced food intake, enhanced lipid metabolism, and further decreased body weight gain(Green & Grieve *et al*., 2018).

2.4.5. Cholecystokinin (CCK)

CCK can be defined as a peptide hormone regarding the gastrointestinal system accountable for stimulating the digestion regarding protein and fat, In the duodenum, the first section of the small intestine, enteroendocrine cells produce and secrete cholecystokinin, also synthesized as pancreozymin (Navarro-Guillén *et al*., 2017). CCK is produced initially as a 115 amino acid preprohormone in intestine and is encoded by the CCK gene on chromosome 3, Following post-translational processing, CCK is transformed into a number of circulating truncated forms, the gastrin receptor, are the specific CCK receptors that when triggered cause the biological actions of CCK peptides (Pathak *et al.,* 2018). The pancreatic digestive tracts enzymes are secreted into the intestine as a result of the gallbladder contracting in reaction to the presence of partially digested food in the duodenum, the hunger is suppressed by this hormone (Rehfeld *et al*., 2019). Cholecystokinin functions as a neurotransmitter in the brain that regulates satiety (H. T. Kim *et al.,* 2021).

CCK also plays significant actions in inflammation, fertility, satiety, cardiovascular function, reduction of gastric acid secretion, which has a good impact

on obesity and glucose metabolism, and stimulation of insulin secretion (Pathak *et al*., 2018).

2.5. Lipid profile

2.5.1. Total Cholesterol (TC)

the lipid cholesterol (C27H46O) was first isolated from human gallstones more than 200 years ago, and there is no denying its pathological and physiological significance, The majority of cells have the ability to synthesize cholesterol, and the liver accounts for roughly half of the total synthesis in humans (Hong *et al*., 2020).

The majority of cholesterol is hydrophobic, just like other sterols, all mammalian cells biosynthesize it, and it is mostly found in cell membranes where it interacts with surrounding lipids for the regulation of the fluidity, rigidity, and permeability of bilayer (Nichols *et al.*, 2019). cholesterol has the ability to bind a variety of transmembrane proteins, maintaining or changing their conformations, Additionally, a variety of sterol transport proteins interact with cholesterol to help with trafficking and regulate its subcellular distribution (Luo *et al*., 2020).

converts excess cholesterol into cholesteryl esters, which are then either released as a key component of plasma lipoproteins such LDLs, VLDLs, and HDLs, or kept as cholesterol reservoir in the cytosolic lipid droplets (Subczynski *et al*., 2017).

2.5.2. Triglycerides (TG)

Triacylglycerol's (TGs), which are often referred to as fat triglyceride, are natural fats comprised of one glycerol molecule connected to three fatty acid molecules (unsaturated/saturated or both) by ester bonds(Laufs *et al.*, 2020).

Those lipids are a crucial component of triglyceride-rich proteins (TRLs), which include chylomicrons, VLDL, and the remaining particles from their catabolism (Ganda *et al*., 2020). The two sources of serum TG are gut absorption and liver syntheses, there are more than 6000 different species of TG, which depend on three fatty acids and their pairings (Pundir & Narwal *et al*., 2018). TGs are important carriers of dietary fats throughout the circulatory system, Chylomicrons and VLDL are largely composed of TGs, in addition to transporting fat, TGs also serve as fat that is stored in adipose tissue and is used when there is a shortage of carbs (Vallejo-Vaz *et al*., 2020).

2.5.3. High-density lipoprotein (HDL)

The densest and smallest lipoproteins in plasma are called HDL, Both ApoA-II and ApoA-I, the two main HDL apolipoproteins, are necessary for typical HDL biosynthesis (Srivastava *et al*., 2018). About 70% of HDL protein is made up of apoA-I, which is synthesized in the liver as well as the intestine and is found on almost all HDL particles, in humans, two-thirds of HDL particles contain apoA-II, which is only synthesized in the liver and accounts for around 20% of HDL protein (Kosmas *et al.*, 2018).

Randomized Controlled Trials (RCT) is the process by which HDL functions as the particular cholesterol acceptor that transports extra cholesterol from peripheral tissues to the plasma and subsequently to liver, in which it could either be excreted directly into the bile or first converted into bile acids or salts, LDL and HDL both significantly contribute to RCT (Ouimet et al., 2019). The three main cardio protective actions of HDL are: (i) preventing LDL from being oxidized; (ii) removal of the cholesterol from the vessel walls and transporting it to the liver; (iii) acting as an anti-inflammatory (Gourgari *et al*., 2019).

According to recent metabolism, HDL cholesterol leads to promoting the secretion of the pancreatic β-cell insulin and modulates the uptake of the glucose in the skeletal muscles (Femlak *et al.*, 2017).

2.5.4. Low-density lipoprotein (LDL)

LDL makes up the majority of your body's cholesterol, HDL, or "good" cholesterol, makes up the remaining portion, LDL is transported by HDL to the liver, in which it is eliminated from the body (Bakke *et al.*, 2020). Since it builds up in the blood vessel walls, the LDL cholesterol is sometimes known as the "bad" cholesterol, increasing the risk of health problem such as a stroke or heart attack, LDL is oxidized within artery walls, contributing to the condition known as atherosclerosis (Lazarte & Hegele *et al*., 2020). However, not all types of the cholesterol are harmful, some are necessary for the body for the purpose of protecting its nerves, producing healthy cells, and hormones (Olesen *et al.*, 2017).

The particles of LDL that having oxidatively changed structural components have been known as the oxidized LDL, Also, LDL's protein and lipid components may both be oxidized in the vascular wall due to free radical attack (Nichols *et al.*, 2019).

2.5.5. Very Low-density lipoprotein (LDL)

The liver produces VLDL cholesterol, which is then released into blood-stream in order to provide the body's tissues with a particular kind of fat (TGs) (Søndergaard *et al.*, 2017). Plaque buildup on artery walls, which narrows and restricts the blood flow pathway, has been related to high levels of VLDL cholesterol (Nakajima & Tanaka *et al*., 2018). VLDL cholesterol cannot be measured simply and easily, which is why it is typically not included in a routine cholesterol check, the proportion of the triglyceride number is typically used to indicate the

VLDL cholesterol level, Over 30 milligrams per deciliter (0.77 mmol/L) of VLDL cholesterol is present (Kornetova *et al.*, 2019). Lowering the triglyceride levels is the most effective approach to lower VLDL cholesterol, the most important things are to exercise frequently and lose weight, you should also avoid alcohol and foods high in sugar, Medications can also help (Balling *et al*., 2020).

Chapter Three
Materials and Methods

Chapter Three

Materials and Methods

3.1. Materials

3.1.1. Chemicals and Kits

Table3-1: Kits and Chemicals that have been utilized in this Study.

No	Kits	Companies	Origin
1.	ABO Reagents	HIMEDIA	India
2.	Cholesterol kit	DIRUI	China
3.	Ethanol	Teeba	Iraq
4.	HbA1c kit	Lifotornic	Germany
5.	HDL kit	DIRUI	China
6.	Human cholecystokinin ELISA Kit	BT LAB	China
7.	Human Ghrelin ELISA Kit	BT LAB	China
8.	Human glutathione ELISA Kit	BT LAB	China
9.	Human Leptin ELISA Kit	BT LAB	China
10.	Human malondialchehyche ELISA Kit	BT LAB	China
11.	Human Obestatin ELISA Kit	BT LAB	China
12.	Iodine	Areej Baghdad	Iraq
13.	LDL kit	DIRUI	China
14.	Random blood sugar Kit	DIRUI	China
15.	Triglyceride kit	DIRUI	China

3.1.2. Tools and Devices

Table 3-2: Tools and Devices of this study.

No	Devices and Tools	Companies	Origins
1.	Auto chemistry analyzer	BIOBASE	China
2.	Centrifuge	ROTOFIX 32 A (Hettich)	Germany
3.	Deep freeze	ALS	Italy
4.	Diposable syring 5 ml	EASYMED	China
5.	Disposal Syringes 10 ml	ULTRA HEALTH	China
6.	EDTA tube	Vaccum blood collection tubes	Iraq
7.	ELx50 Auto strip washer	BIO TEK	U.S.A
8.	ELx800 Auto Reader	BIO TEK	U.S.A
9.	Eppendorf tube	Carl ROTH	Switzerland
10.	Gel tube	Vaccum blood collection tubes	Iraq
11.	Gloves	Mumu plus +	Malaysia
12.	H8 Hemoglobin Analyzer (HPLC)	Lifotronic	Germany
13.	Mask	Disposable 3-layer Mask	China
14.	Micropipettes	Micropipettes	Germany
15.	Refrigerator	LG	South Korea
16.	Tourniquet	Voltaren	China
17.	Vortex	Scientific Industries	Korea

3.2. Methods

3.2.1. Study Design

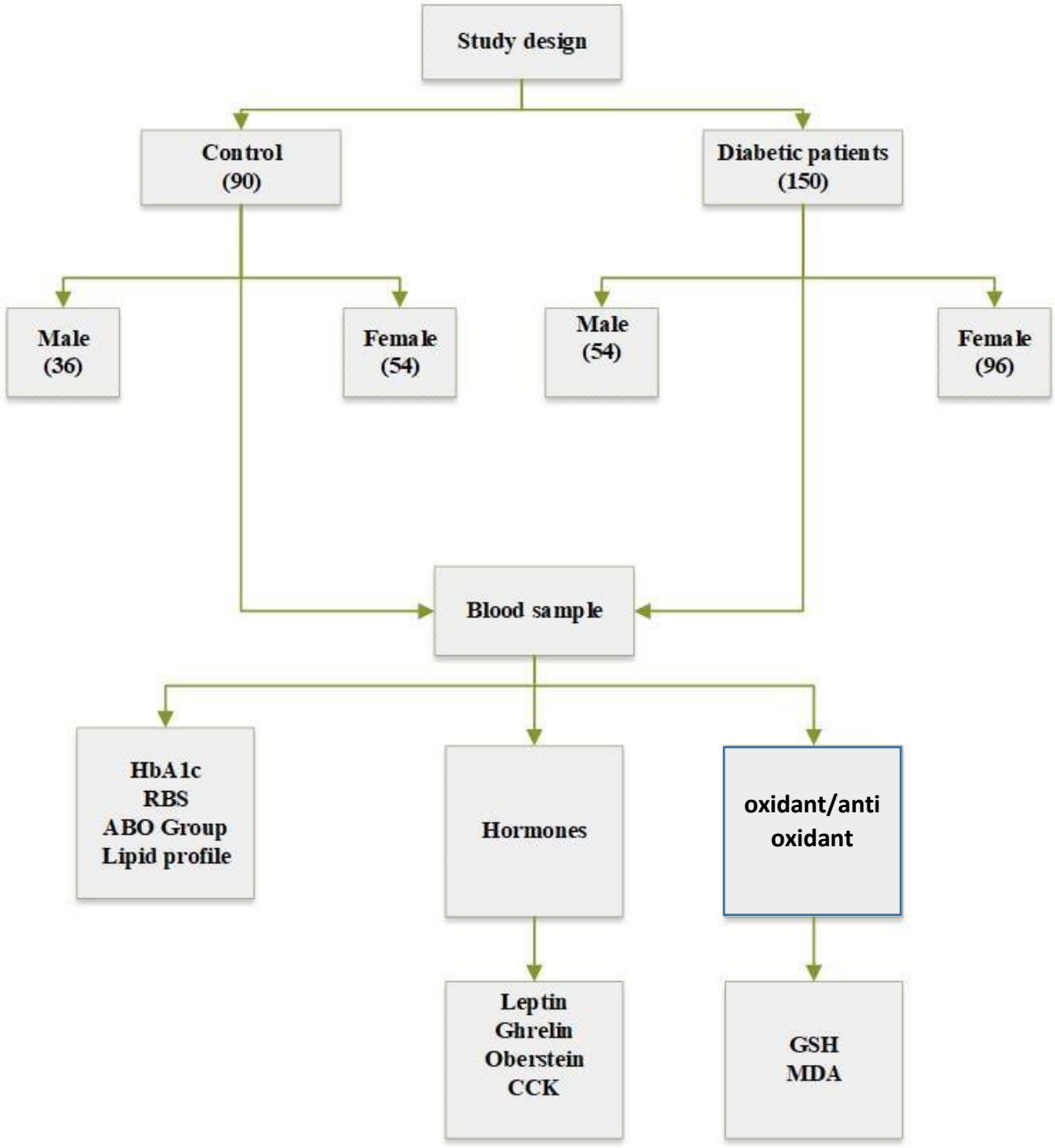


Figure 3-1: Study design

3.2.2. Patients

The current work was designed as case control study, which is also known as "prospective study" and "case-referent study (Creswell & Clark, 2017), Serum samples were collected from 240 participant include 150 patient's cases of type I diabetes mellitus (T1DM), 54 patients (36%) were males and 96 patients (64%) were females and 90 healthy persons, 36 persons (40%) were male and 54 persons (60%) female. got the work done between November 2021 to April 2022 in Karbala, Iraq. The most and main participant were from Al-Hassan centre for diabetes and endocrinology in Al-Husain medical city. The information for each participant was documented according to the questionnaire form, which include age, sex, weight, high, and other questions, as shown in questionnaire form in appendix.

3.2.2.1. Collection Data

All the patients in this study diagnosed of TIDM. The medical history of each patient was taken regarding age, gender, duration of (DM), type of treatment, history of any other complicated diseases, and smoking condition. Measurements of their weight and height have been done for calculating their BMI.

3.2.2.2. Collection samples

With the use of a disposable syringe, five milliliters of venous blood were taken from each participant. Two portions of this blood were separated:

1. The first portion (3 ml) was divided between two gel tubes and allowed to clot at the temperature of the room for about 30 min. The gel tubes were after that centrifuged at 4000 x g in 5 min to get serum, and the first gel tube containing the serum was utilized to automatically evaluate lipid profile and blood glucose. The serum of the second gel tube was put into an Eppendorf tube and stored at a (-20^{o}C) temperature until using it to estimate the

oxidant/antioxidant (GSH and MDA) and hormones (leptin, ghrelin, obestatin and CCK).

2. The second portion of the blood sample (2ml) has been drawn into the EDTA tubes and utilized for the automatic and manual HbA1c and ABO group analysis.

3.2.3. Body Mass Index (BMI) Determination

The BMI has been evaluated via the next equation: $\boldsymbol{BMI}=\boldsymbol{weight}\ (\boldsymbol{kg})/\boldsymbol{height}\ (\text{meters})^2$

For patients and control groups, weight status was categorized according to their BMI as can be seen in table (3) (Lim et al., 2017).

Table 3-3: Weight status was categorized based on their BMI.

Weight Status	WHO (BMI)(kg/m²)
Underweight	Less than 18.50
Normal	18.50 to 24.90
Overweight	25–29.90
Obese	30 or higher

3.2.4. Determination of Rh factor and blood group.

- **Principle**

- The agglutination reaction is the basis for the Rh and ABO blood grouping systems. Red blood cells (RBCs) that contain one of the antigens or both of them interact with each other when they are exposed to appropriate antibodies for the production of a visible agglutination or clumping. The O-linked glycoproteins that make up the ABO blood group antigens are B

or A antigens, depending on the terminal sugar residues that are visible on the RBC surface. People who have A blood group have anti-B serum antibodies and A antigens on their RBCs. B group people also have anti-A antibodies in their serum and B antigens on their RBCs. People with blood group AB have B as well as A antigens on their RBCs but neither B nor A anti-bodies in their serum. People with the blood group O, however, have anti-B as well as anti-A antibodies in their serum, yet neither B antigens nor A antigens. The transmembrane proteins known as Rh antigens interact with the matching antibodies through loops exposed on the surface of RBCs (HiMediaLaboratories, 2015).

❖ Reagents:

Table 3-4: Enlists the Reagents and materials provided in this kit with their quantity and recommended storage.

Contents	Concentration	Storage
Anti RhD Sera	5ml	2-8 ºC
Anti A Sera	5ml	2-8 ºC
Anti B Sera	5ml	2-8 ºC
Mixing stick	300Nos.	RT
Cavity slide	10Nos.	RT
Blood Lancet	100Nos.	RT

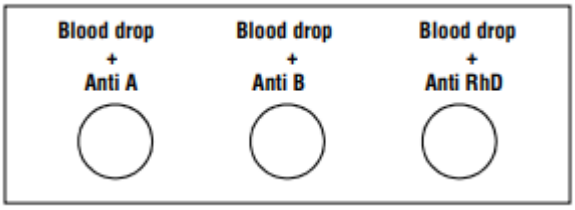
❖ Procedure:

1. Dangle the hand down for increasing blood flow in fingers.

2. Cleanse the fingertip with spirit or 70% alcohol before piercing it (typically middle or ring finger).

3. Pierce the fingertip with the sterile lancet and inject one drop of blood into each cavity.

4. As illustrated below, put one drop of antiserum into each cavity.



2. With the use of new mixing stick, combine the antiserum with each blood drop.
3. Within 30 seconds, look for agglutination in the form of fine red granules. Compared to Anti B and Anti A, Agglutination of Anti RhD takes a little longer.

Note: The lancet and mixing sticks must be disposed of properly.

3.2.5. Determination of HbA1c

HbA1c was measured according to procedure mentioned by Architect Abbott C4000 (Germany).

❖ **Principle**

Total hemoglobin and Glycated hemoglobin (HbA1c) concentrations are each measured separately for the hemoglobin A1c assay. The fraction of the hemoglobin in mmol/mol (IFCC units) or % HbA1c (NGSP units) are calculated using the two concentrations. Only the percent hemoglobin A1c or HbA1c fraction is calculated using the individual concentration values of THb and HbA1c produced by hemoglobin HbA1c or A1c. The anticoagulated whole blood specimen can either be manually lysed with the use of Hemoglobin A1c diluent (A1c DIL) for the hemolysate application or automatically lysed on the system for whole blood application.

❖ Reagents:

Table 3-5: Reagents for determination of HbA1c.

Reactive Ingredients		Concentration
REF	4P52-20 Hemoglobin A1c is supplied as liquid, ready to use	
R1	10-(carboxymethylaminocarbonyl)- 3,7bis (di-methylamino) phenothiazine sodium salt Protease (Bacterial)	0.000817% < 1MU/dl
R2	Peroxidase (Horseradish) Fructose-peptide-oxidase (E. coli, recombinant)	5kU/dl to 15kU/dl 300U/dl to 900U/dl
A1c DIL	Sodium nitrite	>0.05 to <0.30%

❖ Procedure:

1. R1, R2, and A1c DIL were loaded on the ARCHITECT c4000 system.
    - A1c DIL was placed in the reagent supply center for the whole blood application.
    - A1c DIL was configured in the configure reagent setting screen by selecting NEW, then F6 was configured. the reagent name was entered as A1c DIL, the reagent type as sample diluent, the A1c DIL lot number and serial number as shown on the A1c DIL bottle label, and the R1 cartridge size as small (55 ml cartridge).
2. a calibration was performed for the whole blood and/or the hemolysate application.

3.2.6. Measurement of Random Serum Glucose Level

❖ **Principle**

Hexokinase (HK) catalyzes glucose phosphorylation by the ATP for the purpose of forming glucose-6-phosphate and ADP. A second enzyme, glucose-6-phosphate dehydrogenase (G6PDH), has been utilized for catalyzing glucose-6-phosphate oxidation by the NAD + to generate NADH in order to continue the reaction.

$$\text{D-glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{D-glucose-6-phosphate} + \text{ADP}$$

$$\text{D-glucose-6-phosphate} + \text{NADP}^{+} \xrightarrow{\text{G6PDH}} \text{D-6-phosphogluconate} + \text{NADPH} + \text{H}^{+}$$

The produced amount of NADH is directly inversely related to amount of the glucose. The rise in absorbance at 340 nm is used to calculate it .

❖ **Reagents:**

Table 3-6: Reagents for determination of Random Serum Glucose Level.

Contents		Concentration
Reagent 1	MES buffer Mg2+ ATP NADP	5mmol/l 24mmol/L >4.5mmol/L >7mmol/L
Reagent 2	HEPES Buffer Mg2+ HK (yeast) G-6-PDH (E.coli)	200mmol/l 4mmol/L >300ukat/L >300ukat/L

3.2.7. Oxidant/Antioxidant

3.2.7.1. Determination of Human Glutathione Level

❖ **Principle:**

The kit utilized is ELISA. Samples can be added to pre-coated plate. Add the biotinylated antigen next. The biotinylated antigen and antigens in the samples compete for binding to the capture antibody and incubation. In a washing stage, unbound antigen is removed. Next, an avidin-HRP is applied, followed by incubation. Throughout a washing process, unbound avidin-HRP is removed. The addition of TMB Substrate follows, and color results. By adding an acidic stop solution, the process is stopped, and the color changes to a yellow which could be seen at 450 nm. The amount of GSH present in the sample has an inverse relationship with the color developed. The O.D. of the samples is after that compared to the standard curve for determining the GSH's concentration in the sample.

❖ **Reagents:**

Table 3-7: Reagents Provided in Glutathione kit.

Reactive Ingredients	Concentration
Standard/Sample Diluent	6ml × 1 vial
Human GSH Standard, lyophilized	2 vials
Pre-coated Plate	12x 8 well strips x1
Biotinylated Antigen, lyophilized	1 vial
Avidin-HRP Concentrate	100µl x 1 vial
Biotinylated Antigen Diluent	6ml x 1 vial
Wash Buffer Concentrate (25x)	20mlx 1 vial
Avidin HRP Diluent	5.9ml x1 vials
Substrate Solution A	6 ml x 1 vial
Substrate Solution B	6 ml x1 vial
Stop Solution	6 ml x1 vial
Plate Sealer	2 pcs
Zipper Bag	1
User Instruction	1

❖ **Procedure:**

1. As directed, all of the standard solutions, reagents, and samples are prepared. Prior to use, all the reagents are brought to the room temperature. This experiment is carried out at room temperature.
2. Find out the number of the strips that are needed for testing. To use, place the strips in the frames. For up to a month, the unused strips must be kept at 2°–8°C.
3. Blank wells: Add only the stop solution as a blank control, the substrate solution A, and the substrate solution B.
4. 50 µl of the sample should be diluted 2–5 times before being added to the sample well, 50 µl of the biotinylated antigen should be added to each well, and 50 µl of the diluted standard should go in the standard well. Mix well. After sealing the plate, incubate it at 37°C for 60 minutes.
5. Remove sealer and liquid in the well, and then manually wash for 5 times with a 300µl washing buffer. Every time, flip plate over in order to decant the contents; to completely remove the liquid, rap the absorbent material 4-5 times. For automatic washing, aspirate every well before washing five times with wash buffer while overfilling each well. Use an absorbent material to blot the plate.
6. Add 50µl of the avidin-HRP to standard and sample wells, seal the plate, and incubate at 37 °C for 60mins.
7. Wash after removing the sealer as previously mentioned.
8. Each well should first receive 50µl of substrate solution A before receiving 50µl of the substrate solution B. Plate should be incubated for ten mins at a temperature of 37°C in the dark.
9. Each well's color will turn to yellow when 50 µl of Stop Solution are added.

10. Within ten minus of adding stop solution, optical density (OD) of every one of the wells is measured by using microplate reader that has been set to 450nm.

3.2.7.2. Determination of Human Malondialchehyche (MDA) Level

❖ **Principle:**

The utilized kit is ELISA. Human MDA antibody was pre-coated on the plate. When MDA from the sample is introduced, it binds to antibodies which have been coated on wells. Human MDAAntibody which has been biotinylated is after that added, and it binds to MDA in the sample. The biotinylated MDA antibody is after that bound by the addition of streptavidin-HRP. Unbound Streptavidin-HRP is removed throughout a washing step following incubation. Following addition of the substrate solution, color changes according to the concentration of human MDA. By adding an acidic stop solution, the process is stopped, and absorbance has been measured at 450nm.

❖ **Reagents:**

Table 3-8: Reagents Provided in MDA kit.

Reactive Ingredients	Concentration
Standard Solution (80nmol/ml)	0.5 ml x 1
Pre-coated ELISA Plate	12 * 8 well strips x 1
Streptavidin-HRP	6 ml x1
Standard Diluent	3 ml x1
Wash Buffer Concentrate (25x)	20 ml x1
Stop Solution	6 ml x1
Substrate Solution A	6 ml x1
Substrate Solution B	6 ml x1
User Instruction	1
Biotinylated Human MDA Antibody	1 ml x1
Plate Sealer	2 pcs
Zipper bag	1 pc

❖ **Procedure:**

1. As directed, all of the standard solutions, reagents, and samples have been prepared. Prior to use, all of the reagents are brought to room temperature. The experiment is carried out at room temperature.
2. Find out how many strips are required for the testing. To use, the strips are placed in the frames. The strips must be kept between 2° and 8 °C.
3. To the standard well, add 50µl of standard. Note: Since biotinylated antibody is present in the standard solution, do not add it to standard well.
4. 40µl of the sample should be added to the sample wells, which is followed by 10µl of the anti-MDA antibody and 50µl of streptavidin-HRP in the sample as well as the standard wells (Not the blank control well). Mix well, apply a sealant to the plate. At a temperature of 37°C, incubate for 60mins.
5. The sealant is removed, then a wash buffer is used for washing the plate for 5 times. For every one of the washes, the wells are soaked in 300 ul of washing buffer for 30 - 60 seconds. Aspirate or decant thoroughly, then wash five times by washing buffer for automatic washing. Place paper towels or another absorbent material nearby to blot the plate.
6. Every one of the wells should first receive 50µl of the substrate solution A before receiving 50µl of the substrate solution B. Plate should be incubated for ten minus at a temperature of 37°C in the dark.
7. Add 50µl of Stop Solution to every one of the wells, and the blue color will instantly turn into yellow.
8. Within ten minus of adding the stop solution, measure the OD regarding every one of the wells with the use of microplate reader that has been set to 450nm.

3.2.8. Hormones

3.2.8.1. Determination of Human Ghrelin Level

- **Principle:**

The kit that has been used is ELISA. Human GHRL antibody was pre-coated on the plate. When GHRL from the sample is added, it binds to antibodies were been coated on wells. Then, human GHRL anti-body that has been biotinylated is added, and it binds to the sample's GHRL. The biotinylated GHRL antibody is after that bound by the addition of streptavidin-HRP. Unbound Streptavidin-HRP is removed throughout a washing step following incubation. Following substrate solution addition, color develops in a direct proportion to human GHRL concentration. By the addition of an acidic stop solution, the process is stopped, and absorbance is then measured at 450nm (ThermoFisher, 2018).

- **Reagents:**

Table 3-9: Reagents Provided in Ghrelin kit.

Reactive Ingredients	Concentration
Standard Solution (12.8ng/ml)	0.5 ml x1
Standard Diluent	3 ml x 1
Pre-coated ELISA Plate	12 * 8 well strips x1
Stop Solution	6 ml x1
Streptavidin-HRP	6 ml x 1
Wash Buffer Concentrate (25x)	20 ml x1
Substrate Solution A	6ml x 1
Substrate Solution B	6ml x 1
Plate Sealer	2 pcs
Biotinylated Human GHRL Antibody	1 ml x1
User Instruction	1
Zipper bag	1

❖ **Procedure:**

1. As directed, all of the standard solutions, reagents, and samples have been preparing. Prior to use, all the reagents are brought to room temperature. The experiment is carried out at room temperature.
2. Find out how many strips are needed for testing. To use, place strips in frames. The strips must be kept between 2^{o} and 8 °C.
3. To the standard well, add 50µl of standard. Note: Since biotinylated antibody is present in the standard solution, do not add it to standard well.
4. 40µl of sample should be added to the sample wells, followed by 10µl of the anti-GHRL antibody and 50µl of the streptavidin-HRP in the sample as well as the standard wells (Not blank control well). Mix well, apply a sealant to the plate. At a temperature of 37^{o}C, incubate for 60mins.
5. Remove sealant, then use washing buffer to wash plate for 5 times. For every one of the washes, soak the wells in 300 ul of the washing buffer for 30 - 60 seconds. Aspirate or decant thoroughly, then wash for five times using a washing buffer for the automatic washing. Place paper towels or some other absorbent material nearby to blot plate.
6. Every one of the wells should first receive 50µl of the substrate solution A before receiving 50µl of the substrate solution B. Plate should be incubated for ten minus at a temperature of 37^{o}C in the dark.
7. Add 50µl of the Stop Solution into every one of the wells, and the blue color will instantly turn into yellow.
8. Within ten minus of the addition of stop solution, measure OD regarding every one of the wells with the use of a micro-plate reader that has been set to 450nm.

3.2.8.2. Determination of Human Cholecystokinin Level

❖ **Principle:**

The kit used is ELISA. Human CCK antibody was pre-coated on the plate. When CCK from the sample is introduced, it binds to the antibodies that were coated on wells. Human CCK antibody that has been biotinylated is after that added, and it binds to CCK in the sample. The biotinylated CCK antibody is after that bound by the addition of streptavidin-HRP. Unbound Streptavidin-HRP is removed throughout a washing step after incubation. After the addition of the substrate solution, color changes according to the concentration of human CCK. By adding an acidic stop solution, the process is stopped, and absorbance is measured at 450nm .

❖ **Reagents:**

Table 3-10: Reagents Provided in CCK kit.

Reactive Ingredients	Concentration
Standard Solution (128ng/L)	0.50ml x1
Standard Diluent	3ml x 1
Pre-coated ELISA Plate	12x8 well strips x1
Stop Solution	6 ml x 1
Streptavidin-HRP	6 ml x 1
Wash Buffer Concentrate (25x)	20 ml x 1
Substrate Solution A	6ml x1
Substrate Solution B	6ml x1
Plate Sealer	2 pics
Biotinylated Human CCK Antibody	1 ml x 1
User Instruction	1
Zipper bag	1

❖ **Procedure:**

1. As directed, prepare all of the standard solutions, reagents, and samples. Prior to use, bring all of the reagents to room temperature. The experiment has been carried out at room temperature.

2. Find out how many strips are required for testing. To use, place strips in frames. The strips must be kept between 2 and 8ºC.
3. To standard well, add 50µl of the standard. Since standard solution already contains biotinylated anti-body, avoid adding it to standard wells.
4. 40µl of the sample should be added to the sample wells, which is succeeded by 10µl of the anti-CCK anti-body and 50µl of streptavidin-HRP in the sample as well as the standard wells (Not the blank control one). Mixing well, apply a sealant to plate. At a temperature of 37ºC, incubation for 60mins.
5. Remove sealant, then utilize washing buffer in order to wash plate for 5 times. For every one of the washes, soak wells in a minimum of 0.35ml of the washing buffer for 30min to 1 minutes. Aspirate or decant each well for automatic washing, then use wash buffer five times. Place paper towels or some other absorbent material nearby in order to blot the plate.
6. Every one of the wells should first receive 50µl of the substrate solution A before receiving 50µl of the substrate solution B. Plate should be incubated for ten minus at a temperature of 37ºC in the darkness.
7. Add 50µl of the Stop Solution to every one of the wells, and the blue color will instantly turn into yellow.
8. Within 10min after the addition of stop solution, measure OD for every one of the wells with the use of microplate reader that has been set to 450 nm.

3.2.8.3. Determination of Human Leptin Level

- **Principle:**

The kit used is ELISA. Human LEP antibody has been utilized in order to pre-coat the plate. LEP from sample is added, and it binds to antibodies which have been coated on the wells. LEP in the sample is subsequently bound by biotinylated Human

LEP Antibody, which is after that added. The biotinylated LEP antibody is after that bound by the addition of streptavidin-HRP. Unbound Streptavidin-HRP is removed throughout a washing step following incubation. The amount of Human LEP is after that correlated with the amount of substrate solution supplied, and color results. By adding an acidic stop solution, the process is stopped, and absorbance has been measured at 450nm (Biotech, 2010).

❖ Reagents:

Table 3-11: Reagents Provided in Leptin kit.

Reactive Ingredients	Concentration
Standard Solution (12.80ng/ml)	0.50ml x1
Standard Diluent	3 ml x 1
Pre-coated ELISA Plate	12x8 well strips x 1
Stop Solution	6 ml x 1
Streptavidin-HRP	6ml x1
Wash Buffer Concentrate (25 x)	20 ml x1
Substrate Solution A	6 ml x1
Substrate Solution B	6ml x 1
Plate Sealer	2 pcs
Biotinylated Human LEP Antibody	1 ml x 1
User Instruction	1
Zipper bag	1

❖ Procedure:

1. As directed, prepare all of the standard solutions, reagents, and samples. Prior to use, bring all of the reagents to the temperature of the room. The experiment has been carried out at the temperature of the room.
2. Find out how many strips are required for testing. To use, place strips in frames. The strips must be kept between 2^{o} and 8 °C.
3. To the standard well, add 50µl of standard. Since biotinylated antibody is present in the standard solution, do not add it to standard well.

4. 40µl of sample should be added to the sample wells, succeeded by 10µl of the anti-LEP antibody and 50µl of the streptavidin-HRP in the sample as well as the standard wells (Not the blank control one). Mixing well, apply a sealant to plate. At a temperature of 37^{o}C, incubate for 60mins.
5. Remove sealant, then utilize a washing buffer for washing the plate for 5 times. For every one of the washes, soak the wells in 300.0ul of the washing buffer for 30sec to 60 seconds. Aspirate or decant thoroughly, then wash for five times by using the washing buffer for the automatic washing. Place paper towels or some other absorbent material nearby to blot plate.
6. Every one of the wells should first receive 50µl of the substrate solution A before receiving 50µl of the substrate solution B. Plate should be incubated for ten minus at a temperature of 37^{o}C in the darkness.
7. Add 50µl of the Stop Solution to every one of the wells, and the blue color will instantly turn into yellow.
8. Within ten minus of the addition of stop solution, measure OD regarding every one of the wells with the use of a microplate reader set to 450 nm.

3.2.8.4 Determination of Human Obestatin Level

- **Principle:**

The kit used is ELISA. Human OB antibody was pre-coated on plate. The sample's OB has been introduced, and it binds to antibodies which were coated on wells. Human OB anti-body that has been biotinylated is added after that, and it binds to OB in the sample. The biotinylated OB antibody is after that bound by the addition of streptavidin-HRP. Unbound Streptavidin-HRP is removed throughout a washing step following incubation. After the addition of the substrate solution, color changes

according to the concentration of human OB. By adding an acidic stop solution, the process is stopped, and absorbance has been measured at 450nm .

❖ Reagents:

Table 3-12: Reagents Provided in Obestatin kit.

Reactive Ingredients	Concentration
Standard Solution (640ng/L)	0.50ml x1
Standard Diluent	3ml x 1
Pre-coated ELISA Plate	12x8 well strips x 1
Stop Solution	6 ml x 1
Streptavidin-HRP	6 ml x1
Wash Buffer Concentrate (25 x)	20ml x 1
Substrate Solution A	6 ml x1
Substrate Solution B	6 ml x 1
Plate Sealer	2 pcs
Biotinylated Human OB Antibody	1 ml x1
User Instruction	1
Zipper bag	1

❖ Procedure:

1. As directed, prepare all of the standard solutions, reagents, and samples. Prior to use, all the reagents are brought to room temperature. The experiment has been carried out at the temperature of the room.
2. Find out how many strips are needed for the test. To use, the strips are placed in frames. The strips must be kept between 2º and 8 °C.
3. To the standard well, add 50µl of the standard. Since biotinylated antibody is present in the standard solution, do not add it to standard well.
4. 40.0µl of the sample should be added to the sample wells, succeeded by 10µl of the anti-OB antibody and 50µl of the streptavidin-HRP for the standard wells (Not the blank control one). Mix well, apply a sealant to plate. At a temperature of 37ºC, incubate for a duration of 60mins.

5. Sealant is removed, then a washing buffer is used in order to wash plate for 5 times. For every one of the washes, the wells are soaked in 300.0ul of the washing buffer for 30sec to 60 seconds. Aspirate or decant thoroughly, then wash for five times by a washing buffer for the automatic washing. Place paper towels or some other absorbent material nearby to blot plate.
6. Every one of the wells should first receive 50µl of the substrate solution A before receiving 50µl of the solution B. Plate should be incubated for ten minus at 37°C in the darkness.
7. Add 50µl of the Stop Solution into every one of the wells, and the blue color will instantly turn to yellow.
8. Within ten minus of the addition of the stop solution, determine OD regarding every one of the wells by the use of a microplate reader set to 450nm.

3.2.9. Estimate of Lipid Profile

3.2.9.1. Total Cholesterol (TC)

- **Principle:**

Cholesterol oxidase and cholesterol esterase are used in an enzymatic, colorimetric technique.

Cholesteryl esters + H2O ⟶ CHE cholesterol + fatty acids

Cholesterol+O2 ⟶ CHO cholest-4-en-3-one +H2O

2H2O+4aminoantipyrin+phenol ⟶ POD quinoneimine dye+ 4H2O

(red coloured)

The concentration of cholesterol correlates with the intensity of the color (Bachorik & Ross, 1995)

3.2.9.2. Triglycerides (TG)

❖ **Principle:**

glycerophosphate oxidase is used in an enzymatic, colorimetric technique.

Triglycerides+H2O ⟶ LPL glycerol +fatty acids

Glycerol +ATP ⟶ GK glycerol-3-phosphate+ATP

glycerol-3-phosphate+O2 ⟶ GPO dihydroxy-acetone-phosphate+$2H_2O_2$

$2H_2O_2$+4-AA+ADPS ⟶ POD quinoneimine dye+4H2O

The quantity of TGs present affects the color's intensity. The reagents, stored at a temperature of 2^{o}-8^{o}C are stable until expiration date that has been written on the packaging. The reagent stored on board of the analyzer at 2-10°C is stable for 10 weeks (ACCENT-200) or for 12 weeks (ACCENT MC320).

3.2.9.3. High Density Lipoprotein (HDL)

❖ **Principle:**

without any off-line pretreatment or centrifugation processes, the assay offers homogenous approach for the direct measurement of the HDL-cholesterol levels in the plasma or serum. Methodology for using an accelerator-selective detergent. Throughout the first phase, VLDL, LDL particles, and Chylomicrons release free non-HDL cholesterol that produces hydrogen peroxide via an enzymatic process. A peroxidase reaction with N,N-bis(4sulphobutyl) m-toluidine-disodium (DSBmT) consumes the produced peroxide, resulting in a colorless product. HDL-Cholesterol is solubilized during the second phase using a particular detergent. Peroxidase and ***4***-aminoantipyrine(4-AAP) produce a colored response that is proportionate to HDL-Cholesterol content when they work along with cholesterol oxidase (CO) and cholesterol esterase (CE).

Accelerator + CO

VLDL, LDL, Chylomicrons Non-Reactive LDL, VLDL, Chylomicrons ⟶

DSBmT + Peroxidase

cholesterol esterase

HDL-C + Selective Detergent cholestenone + H_2O_2 ⟶ cholesterol oxidase

Peroxidase

H_2O_2 + 4-AAP ⟶ (color development) DSBmT

3.2.9.4. Low Density Lipoprotein (LDL)

- **Principle:**

Without any off-line preparation or centrifugation, the test provides a homogenous approach for detecting LDL-cholesterol concentrations in plasma or serum. Method using a liquid selective detergent. The approach is based on the characteristics of a single detergent and uses two reagents. Only the non-LDL particles are solubilized by this detergent (Reagent 1). (HDL, VLDL, CM). Cholesterol oxidase and cholesterol esterase consume the generated cholesterol in a non-color generating reaction. The remaining LDL particles are solubilized by a second detergent (Reagent 2), and a chromogenic coupler enables for color formulation. The amount of LDL cholesterol in the sample is reflected in the color created by the enzyme reaction with LDL cholesterol with the existence of coupler.

Cholesterol (TC), TG, HDL-C, and LDL have been all automatically tested in BIOBASE auto chemistry analyzer followed an approach of .

3.3. Ethical management of studies

The research followed the guidelines set forth by Dept. of Clinical Laboratories at University of Karbala's College of Applied Medical Sciences for dealing with biological substances. After acquiring the necessary authorization from the hospital administration and patients, the samples in this work have been collected from the patients who arriving at Al-Hassan center for diabetes and endocrinology in Al-Husain medical city at Kerbala Governorate/ Karbala Health Directorate.

3.4. Statistical analysis

The version twelve of the computer program, SPSS, which has been utilized for data analysis. the data have been represented as mean standard deviation (± Sd). they were estimated differences among groups via using T test with the P value (i.e., the least significant difference) has been found for the comparison amongst the groups, and the results have been considered to have statistical significance at ($p \leq 0.05$)

Chapter Four

Results and Discussion

Chapter Four

Results and Discussion

4.1. General parameters

4.1.1. Age

According to the results in table (4-1) showing no significant differences (P > 0. 05) in age group less than 5 years and age group (42-50). There were high significant differences (P≤0.001) in two age group (6-11) and (12-17) also a significant difference (P≤ 0.05) in age group (18-23), (24-29), (30-35) and (36-41), as observed in table (4-1). Also results clarified that the more age group susceptible and affected by T1DM is (12-17) with a percentage of 48%. While the age groups less than 5 years and a group (42-50) it is the little patients recoded with T1DM at percentage 1.33% and 2.67%.

Based on the results of table (4-2), there have been non-significant decrease (P ≥ 0.05) in the age data in T1DM male's patients compared to controls, as the mean (17.592 and 22.33). The results of the same table had revealed high significance decrease (P ≤ 0.001) for average of age between females in both groups (17.833 and 27.555).

Table4-1: Distribute the group of patients with T1DM according to specific age groups and compare them with a control group.

Age (Years)	Patients		Control		Total	P_{value}
	N	%	N	%		
>5	2	1.33	6	6.67	8	0.1573
6 – 11	20	13.33	---		20	0.00001 **
12 – 17	72	48	12	13.33	84	0.00001 **
18 – 23	24	16	6	6.67	30	0.00102 *
24 – 29	18	12	42	46.66	60	0.00195 *
30 – 35	6	4	18	20	24	0.01431 *
36 – 41	4	2.67	---		4	0.0455 *
42 – 50	4	2.67	6	6.67	10	0.52709
Total	**150**	**100**	**90**	**100**	240	0.00011 **

* indicates the significance difference ($p \leq 0.05$) ** indicates the high significance difference ($p \leq 0.001$)

Table4-2: The relationship between age and T1DM according to gender in the patient group and its comparison with the control group

Age (years)	Patients		Control		P value
	N (%)	Mean ± SD	N (%)	Mean ± SD	
Male	54(36%)	17.592±10.244	36(40%)	22.33 ± 12.225	0.3288
Female	96(64%)	17.833±6.99	54(60%)	27.555 ± 8.293	0.0005 **
Total	150(100%)	17.746±8.24	90(100%)	25.466±9.984	0.0001 **

** indicates the high significance difference ($p \leq 0.001$)

Many studies have addressed and supported the findings of the present investigation, which shows that T1DM was strongly correlated with age, particularly in adolescence and infancy as shown in table 4-1, and founded relation between age and T1DM according to gender. According to a recent analysis by the Number of Patients with T1DM in 2017, there are an estimated 1,106,500 million young people (under 20) living with T1DM globally, which is twice as many as there were in 2015 (Robert et al., 2018). Incidence was lowest in youngest (1-4) and oldest

(15-19) age groups, and it was highest in children aged 5 to 14 years, The incidence increased most significantly in children who were 10 to 14 years old (Fox et al., 2018). agreement with other studies, a 60-year review of data from the UK Biobank indicates that up to half of all incidence cases of T1DM were identified as adults, Because of the difficulty in identifying T1DM from T2DM requiring insulin therapy and the possibility that more than 20% of individuals with T1DM are also receiving insulin, incidence rates in adult populations are rarely reported (Gajewska et al., 2020). Based on one of the recent analyses, Iraq revealed a high frequency of T1DM as well, with over 8,000 adolescents and children suffering in 2019, When compared to other middle eastern nations, a moderate increase in prevalence and incidence of T1DM has been seen (Alhazmi et al., 2020). T1DM was reported to affect adults more frequently than previously thought in Western countries (Lee et al., 2019). Diabetes prevalence and incidence are rising globally, The IDF projected those 425 million persons who were aged between 20 and 79 had diabetes in 2017 (All types), Even though T1DM has generally been referred to as "juvenile diabetes" and is thought to affect children, new research indicates that it may really affect adults more frequently than previously thought, About a quarter of people with T1DM are adults, and adults ≥ 20 make up more than a million (or 85%) of all type diabetes cases in the US (Saeedi et al., 2019).

4.1.2. Gender

The results of table (4-3) revealed a significant difference ($P \leq 0.05$) between males in both groups and high significant difference ($P \leq 0.001$) between females in both groups (patients and control). Also indicated the percentage of females (64%) is higher than the percentage of males (36%) in T1DM group.

Table 4-3: Distribution the group of patients with T1DM according to gender and compare them with a control group.

Genders	Patients N (%)	Control N (%)	Total	P_{value}
Male	54(36%)	36(40%)	90(%)	0.05778 *
Female	96(64%)	54(60%)	150(%)	0.00061 **
Total	**150(100 %)**	**90(100 %)**	**240(100%)**	0.00011 **
P value	**0.00061 ****	**0.05778 ***	**0.00011 ****	

* indicates the significance difference ($p \leq 0.05$) ** indicates the high significance difference ($p \leq 0.001$)

Table (4-3) in the most recent research clearly demonstrates that women suffer from T1DM the most, as seen in Figure (4-1) from earlier researches that most closely align with the current work. In comparison to men, women were noticeably more possibly to report using an insulin pump (Shah et al., 2018). In the case of comparing the rates in the females and males in 2002 - 2003 compared to 2012 - 2013, it seems that an increase in the incidence has been higher in the male patients compared to it in the female patients, on the other hand, has been higher in the female, It is significant to note that incidence that was reported reflected one year, while APC reflected the trends of the incidence throughout the 11-year period, Males had a consistently higher T1DM prevalence compared to the females (Fox et al., 2018). The work had 75 participants, of which 39 were female and 36 were male, women made up the majority of the study's participant population (Malik et al., 2018). Another study of 214 adolescents (aged 13 to 18) with the T1DM in the central regions of the Saudi Arabia revealed that the female gender, longer T1DM durations and numerous daily injections were associated with females (Robert et al., 2018).

4.1.3. Weight and Body Mass Index (BMI)

The findings of the statistical analysis for weight, as showing in table (4-4), revealed insignificant decrease ($P \geq 0.05$) in the weight value in T1DM of male's patients compared to controls, as average (50.407 and 58.666), in the same table revealed significant decrease ($P \leq 0.05$) of the mean of weight for T1DM of female's patients compared to controls (50.416 and 57.666), while showed no significant differences ($P \geq 0.05$) between females and males in patient group control group.

The values of BMI for males and female in T1DM patients demonstrated an insignificant decrease when comparison with control group. Additionally, there was a significant decline ($P \leq 0.05$) in the mean BMI of total T1DM patients compared with the control group (33.128and 35.62).

Table 4-4: Data of body mass index and weight in control and T1DM patients and compare with control.

Weight		Mean ± SD(Kg)	Mean ± SD (Kg)	
	Male	50.407±22.472	58.666±23.94	0.0997
	Female	50.416±16.492	57.666±6.419	0.0023 *
	Total	50.413±18.852	58.066±15.816	0.0014 **
	P value	0.9978	0.7707	
BMI		Mean ± SD (Kg/m)	Mean ± SD (Kg/m)	
	Male	32.553±11.467	35.516±9.677	0.2053
	Female	33.451±9.516	35.69±3.007	0.0950
	Total	33.128±10.267	35.62±6.498	0.0398 *
	P value	0.6076	0.9018	

* indicates the significance difference ($p \leq 0.05$) ** indicates the high significance difference ($p \leq 0.001$)

According to our research, patients with T1DM have decreased weight, length, and BMI as show in table (4-4). DM is linked to numerous metabolic abnormalities within the body, such as indigestion of carbohydrates that results in

weight loss and malnutrition (Patel et al., 2006). Previous researches also showed a reduction in BMI and weight, Women with T1DM had a markedly higher BMI than men did (Maiorino et al., 2018). Female gender was connected with increased BMI, according to data from diabetes follow-up registry in Austria and Germany (Shah et al., 2018). A collection of diseases collectively indicated as diabetes are characterized by elevated blood glucose levels, Protein and lipid metabolic diseases are the result of a shortage in the generation or action of insulin, or the two, which might happen for a variety of causes, Hypoglycemia's long-term effects include tissue and organ damage, Diabetes can cause thirst, polyuria, eye problems, and weight loss (Norris et al., 2020)

4.2. Physiological parameters

4.2.1. Blood Group

Table (4-5) was showed there have been no significant differences ($P \geq 0.05$) in blood group A and AB in both control and patient groups. Furthermore, high significance differences ($P \leq 0.001$) were observed in blood groups B and O when compared between controls and patients with type I DM, and high significance differences ($P \leq 0.001$) in all blood groups of controls and patients with type I DM, separately.

Table 4-5: The percentage of blood groups of the patients of TIDM compare control group.

Blood Groups	patients	Control	Total	P value
A	47.5% 38 25.34%%	52.5% 42 46.66%	80	0.65472
B	81.25% 26 17.33%	18.75% 6 6.67%	32	0.00041 **
AB	70% 14 9.33%	30% 6 6.67%	20	0.07364
O	66.67% 72 48%	33.33% 36 40%	108	0.00053 **
Total	150	90	240	0.00011 **
P value	0.00001 **	0.00001 **	0.00001 **	

** indicates the high significance difference ($p \leq 0.001$)

According to table (4-5)'s findings, there were notable differences in the ABO group with T1DM, especially in groups O and B. Additionally, the results of a previous research revealed that blood group B was the most prevalent in Jodhpur City, succeeded by O, A, and AB in both females and males as well as general population, Additionally, the highest prevalence of diabetes has been discovered in blood group B, which was after that followed by O, A, and AB in both females and males as well as the general population (Sharma et al., 2014). According to the ABO blood type distributions, significantly more pancreatic cancer patients than control patients had been diagnosed with diabetes (Li et al., 2018). Results from the chi-square test indicated a negative or inverse relationship between the ABO blood groups and T1DM, and the O blood groups also revealed a negative relationship with DM, suggesting that those with the A and O blood types are less likely to develop T1DM, Yet, no connection between T1DM and blood types AB and B was

discovered, In 140 samples from healthy controls, blood group A has been the most common, followed by group A, Patients with T1DM had a high frequency of B blood group followed by the O blood group (Kamil et al., 2010)

4.2.2. HbA1C

Table (4-6) showing there were high significant increase ($P \leq 0.001$) in average of HbA1C in patients who have the T1DM compared with the control groups, HbA1C rate for the patients who have T1DM and the controls (10.345 & 5.206). As well as, there was no significant differences ($P \geq 0.05$) in both groups' patients and controls according to gender.

Table 4-6: Distribute the group of patients with T1DM according to HbA1c and compare them with a control group

HbA1C	Patients	Control	P_{value}
	Mean ± SD	Mean ± SD	
Male	10.1±2.062	5.283±0.466	0.0001 **
Female	10.483±2.649	5.155±0.247	0.0001 **
Total	10.345±2.462	5.206±0.354	0.0001 **
P_{value}	0.3606	0.0935	

** indicates the high significance difference ($p \leq 0.001$)

Results in table (4-6) revealed that diabetic patients' mean HbA1c values significantly increased compared to control group. this finding is relevant to the current investigation, HbA1c levels were greater in T1DM patients than in controls, as expected (Karaca et al., 2019). when compared with the controls, patients who have T1DM had significantly higher levels of HbA1c, fasting glucose, and total cholesterol (Wędrychowicz et al., 2019). Women with T1DM exhibited higher HbA1c levels, BMI, HDL cholesterol, and a lower count of CD34+KDR+CD133+ as well as CD133+KDR+ than males did, Additionally, more women than men had

obesity or overweight, smoking, thyroiditis, and sexual dysfunction (Maiorino et al., 2018).

4.2.3. Glucose

The concentration of glucose in T1DM patients was significantly higher ($P \leq 0.001$) than controls, according to the results of table (4-7) the mean of glucose for T1DM patients and controls is (222.464 and 101.4) mg/dl, In the same table also indicated insignificant reduction ($P \geq 0.05$) in males' comparison to females in patients' group (217.74 and 225.12) mg/dl and in controls group (100.166 and 102.222) mg/dl.

Table 4-7: Concentration of glucose in patients with TIDM and compare with control group

Glucose	Patients	Control	P_{value}
	Mean ± SD (mg/dl)	**Mean ± SD (mg/dl)**	
Male	217.74±119.585	100.166±12.632	0.0001 **
Female	225.12±103.272	102.222±8.667	0.0001 **
Total	222.464±109.451	101.4±10.722	0.0001 **
P value	0.6922	0.3619	

** indicates the high significance difference ($p \leq 0.001$)

In current study the results have shown an increase in glucose levels in patients relative to controls subjects, which is consistent with many previous studies. In some study that supports current results the ratio of glucose concentrations in patients to healthy subjects was very high according to the statistical analysis ((*Kolodziejski et al.*, 2017). Low levels of blood insulin and high levels of the blood glucose (i.e. hyperglycemia) are also linked to T1DM, Conversely, diabetes is characterized by high FBG (FPG ≥ 126 mg/dL) (Sudirman *et al.*, 2019). Hyperglycemia in the blood,

which results from inadequate pancreatic insulin secretion or insufficient insulin-directed glucose uptake by target cells, is the primary sign of DM (B *et al*., 2018). A lengthy latency period, which reflects the huge number of functional β cells which must be eliminated prior to the clinical manifestation of the disease, precedes the onset of symptomatic hyperglycemia (Paschou *et al*., 2018). According to data from diabetes follow-up registry in Austria and Germany, women were more likely to have poor glycemic control, an increased total cholesterol, BMIs, and LDL cholesterol (Shah *et al.*, 2018).

4.3. Oxidant/Antioxidant

4.3.1. Malondialdehyde (MDA)

The results of the MDA levels in both patient and control groups is presented in table (4-8) was found a significant increase ($P \leq 0.05$) in the concentration of MDA for patients with T1DM when compared with the control group, the concentration of MDA for male's patients with T1DM and the control is (12.135 and 7.991) nmol/mL, also a significant increase ($P \leq 0.05$) in the concentration of MDA for female's patients with T1DM comparison with the control groups as the concentration of MDA for female's patients with T1DM and the control is (11.51 and 9.343) nmol/ml and a significant increase ($p \leq 0.05$) in the concentration of MDA of patients with T1DM comparison to the control groups as the concentration of MDA is (11.735 and 8.802) nmol/ml, . lastly showing no significant difference in concentration of MDA between males and females in both patients and controls groups.

Table 4-8: Concentration of MDA in patients with TIDM and compare with control group

MDA	Patients	Controls	P_{value}
	Mean ± SD nmol/mL	Mean ± SD nmol/mL	
Male	12.135±7.985	7.991±4.274	0.0054 *
Female	11.51±8.685	9.343±4.791	0.0926
Total	11.735±8.418	8.802±4.615	0.0026 *
P_{value}	0.6640	0.1747	

* indicates the significance difference ($p \leq 0.05$)

In the case when put to comparison with control groups, the data in table (4–8) showed a substantial rise in MDA concentration in the patient groups. These findings are consistent with certain researches that suggested a rise in MDA levels. A research that included 40 children who have the T1DM and 40 healthy controls found that the level of MDA (a measure of lipid peroxidation) that indicates an increase in the oxidative stress in the diabetics as it has been described in other studies, was significantly higher in the children with T1DM (Amrousy *et al.*,2020). MDA, a sign of increased lipid peroxidation, was detected in diabetic individuals' RBC membranes, When put to comparison with non-diabetic people, the plasma regarding diabetic subjects has higher circulating levels of MDA (Jalees & Rosaline, 2017). Lipid peroxidation (LPO) is regarded as a marker of oxidative damage to tissues and cells, several decades ago, observed that, in accordance with other more recent research, plasma LPO levels of DM patients with angiopathy are greater than those of the general population and are higher than those of DM patients without any problems (Chatziralli *et al.*, 2017). MDA concentrations have been considerably higher in the blood of T1DM individuals than the corresponding control group, according to research by Alghazeer (Alghazeer *et al*., 2018). Additionally, diabetic males' levels of NO and MDA were marginally (but not substantially) higher than diabetic females' levels; these findings are consistent with recent findings,

According to other research, the lipid peroxidation biomarker (MDA) has been considerably higher in both groups (patients who have existing T1DM and patients with newly diagnosed T1DM) in comparison with the healthy group, which explains why serum MDA is likely connected with T1DM (Abdel-Moneim *et al*., 2020). Even in people with well-controlled diabetes, dyslipidemia seen in the diabetes might have a significant effect on systemic inflammation through altered oxidative metabolism, resulting in increased lipid peroxidation (MDA) (de Souza Bastos *et al*., 2016). Throughout the initial observation period, MDA was positively correlated with poorer diabetes control as measured by a higher HbA1c, which is consistent with prior results (Kostopoulou *et al.*, 2020).

4.3.2. Glutathione (GSH)

The results of GSH in table (4-9) was found a significant decrease ($P \leq 0.05$) in concentration of GSH in male's patients of T1DM when compared with control group, the concentration of GSH for patients with T1DM and the control is (7.745 and 10.961) nmol/mL, and a significant decrease ($P \leq 0.05$) in concentration of GSH in female's patients when compared whit control group, the concentration of GSH for patients with T1DM and the control is (7.045 and 9.243) nmol/ml , also high significant decrease ($p \leq 0.001$) in concentration of GSH in patients with T1DM when compared with the control group, the concentration of GSH is (7.325 and 9.861) nmol/ml lastly the result appears no significant differences ($p \geq 0.05$) in concentration of GSH between male and female patients group.

Table 4-9: Concentration of GSH in patients with TIDM and compare with control group

GSH	Patients	Control	P_{value}
	Mean ± SD nmol/mL	Mean ± SD nmol/mL	
Male	7.745±1.627	10.961±6.047	0.0025 *
Female	7.045±4.433	9.243±3.94	0.0021 *
Total	7.325±3.586	9.861±4.857	0.0001 **
P value	0.3673	0.0371 *	

* indicates the significance difference ($p \leq 0.05$) ** indicates the high significance difference ($p \leq 0.001$)

The concentration of GSH in T1DM patients was significantly lower in this work; the findings have been consistent with previous investigations that also found a reduction in GSH concentration. GSH imbalance has been seen in a variety of pathological situations, including tuberculosis (TB), diabetes, HIV, aging, and cancer, as GSH deficit increases the risk of oxidative damage to cells (Teskey *et al*., 2018). Children with T1DM, particularly those with poorly controlled cases, showed low serum GSH that was statistically substantially lower in diabetic group than in the controls, which may have an impact on the erythrocyte GSHGPx system (Alghobashy *et al*., 2018). In a different study, (Malik *et al.*, 2018) described their findings in the following way: In T1DM patients with illness duration five years compared with disease duration, the levels of decreased GSH have been lower and the levels of catalase and superoxide dismutase have been higher. Children who have T1DM had considerably lower SOD and glutathione levels in comparison with their respective values in controls, whereas MDA levels have been considerably higher in the children who are T1DM in comparison to the control group, according to recent results that were also similar results for GSH observed in a study that has been conducted by El Amrousy (Amrousy, 2020). The level of GSH has been observed to be significantly decreased in the diabetic men. GSH, on the other hand, was only elevated in diabetic females (Alghazeer *et al.*, 2018).

4.4. Hormones

4.4.1. Leptin

The result of table (4-10) demonstrated that no any significant differences in the leptin concentration of male's patients as compared to the control group, the concentration of leptin for patients with T1DM and the control is (2.963 and 2.443) nmol/mL, In the same table showed high significant increase ($P \leq 0.001$) in concentration of leptin in female's patients when compared with control group, the concentration of leptin for female's patients with T1DM and the control is (3.059 and 2.029) nmol/ml, also high significant increase ($P \leq 0.001$) in concentration of leptin in patient's comparison to controls, the concentration of leptin for patients with T1DM and the control is (3.024 and 2.194) nmol/mL, lastly showing no significance differences ($p \geq 0.05$) in concentration of leptin between males and females in patients group while there is significant increase ($P \leq 0.05$) in male's comparison females in controls group as the concentration of leptin (2.443 and 2.029) nmol/mL.

Table 4-10: Concentration of Leptin in patients with TIDM and compare with control group

Leptin	Patients	Controls	P_{value}
	Mean ± SD nmol/mL	Mean ± SD nmol/mL	
Male	2.963±1.264	2.443±1.37	0.0678
Female	3.059±1.492	2.029±0.471	0.0001 **
Total	3.024±1.411	2.194±0.955	0.0001 **
P_{value}	0.6905	0.0432 *	

* indicates the significance difference ($p \leq 0.05$) ** indicates the high significance difference ($p \leq 0.001$)

The serum leptin level in this work's diabetic patients has been significantly higher compared to it in non-diabetic patients, and there have not been any significant gender differences in the T1DM patients. Particularly throughout pubertal development, the leptin data of T1DM patients in comparison with healthy controls are contentious. It has also been discovered that there is a relationship between diabetes HbA1c level and leptin level. The most recent findings contradict (Ismail *et al*., 2017), who claimed that there were no appreciable variations in leptin or adiponectin levels between control and diabetic patients. According to a different study (Kapustin *et al.*, 2020), following comparing the leptin levels regarding pregnant women in groups with the three forms of diabetes and a group of healthy women, they found that, in contrast to the controls, all other groups had high levels of leptin in third trimester. Leptin concentrations measured in the morning tended to be higher in T1DM patients in comparison with controls, yet were in the same range with a slight increase in the two groups after lunch, The concentration of leptin significantly reduced in the both controls and patients following breakfast and returned to initial concentrations around lunch time (Trefz *et al.,* 2019). Disagreement with (Abd El Dayem *et al.,* 2017), who discovered that median (IQR) serum leptin level has been considerably lower in diabetic, overweight group II (5.2ng/ml - 24.8ng/ml) in comparison with non-diabetic, overweight group. This was explained by the possibility that the lower leptin concentrations in newly diagnosed T1DM patients may be brought on by an inadequate supply of insulin and increased lipolysis. Although there has not been any statistically significant difference between those two groups in terms of leptin or adiponectin levels, patients with T1DM had somewhat higher levels than controls (Wędrychowicz *et al*., 2019). Both fasting and T1DM cause a significant decrease in leptin (Xu & Tong, 2017). Those pathological conditions imply that the insulin results in stimulating the release of the leptin because children with new-onset T1DM and patients with insulinomas

have high and low plasma leptin levels, , Insulin therapy raises the levels of the leptin within 24 hours, reaching non-diabetic levels by 3 to 5 days, whereas the removal of the tumor restores plasma leptin to the normal levels (Marques-Oliveira *et al.*, 2018). Serum leptin levels in humans have been shown to positively correlate with BMI, body fat percentage, adipocytes' size and fat mass, Obesity-related enlargement of the adipocytes in the humans leads to accelerated leptin secretion and higher serum leptin levels that could result as well from the increased turnover of the cortisol and chronic hyperinsulinemia (Marques-Oliveira *et al.*, 2018). Leptin is influenced by hormonal and metabolic factors such as steroid, insulin, thyroid, and estradiol, which stimulate, and testosterone, which inhibits leptin production. Its lack or resistance causes obesity, hyperphagia, and DM (Onyemelukwe *et al*., 2020).

4.4.2. Ghrelin

Results of statistical analysis in table (4-11) for ghrelin appears there were no significant differences ($P \geq 0.05$) in concentration of ghrelin in male patient's comparison to male controls (2.415 and 2.391) nmol/mL, and significance increase ($P \leq 0.05$) in concentration of ghrelin in female patient's comparison to controls (2.403 and1.938) nmol/mL, also there were significance increase ($P \leq 0.05$) in concentration of ghrelin for T1DM patients and the control.

Table 4-11: Concentration of Grelinin patients with TIDM and compare with control group

Ghrelin	Patients	Controls	P_{value}
	Mean ± SD nmol/mL	Mean ± SD nmol/mL	
Male	2.415±1.18	2.391±0.766	0.9145
Female	2.403±1.344	1.938±0.517	0.0159 *
Total	2.411±1.237	2.119±0.663	0.0398 *
P_{value}	0.9564	0.0012 *	

* indicates the significance difference ($p \leq 0.05$)

The findings in tables (4–11) provide evidence that there is a connection between T1DM and ghrelin. Other researches showed that ghrelin levels rise prior to meals and fall following them (Warchoł *et al*., 2018). In comparison to non-diabetic controls and T1DM controls, the levels of ghrelin have been lower in the non-diabetic pregnant and T1DM pregnant women , Both T1DM diabetic and non-diabetic women's lower ghrelin levels throughout pregnancy indicate that the ability to control one's appetite is impacted (Nalla *et al*., 2020). Despite studies suggesting that the levels of ghrelin fall with the beginning of T1DM, ghrelin also has protective effects on the β-cells by protecting them from programming death under conditions of T1DM (Poher *et al*., 2018). They have noticed as well that the obesity could affect ghrelin levels because plasma ghrelin concentration levels have been lower in the patients with obesity, and that indicates that the obesity could impact regulations regarding production of ghrelin, Plasma ghrelin concentrations have been lower in the diabetic patients with poor long-term glycemic control compared to patients with the good long-term glycemic control (Özcan *et al*., 2021). Since ghrelin has also been demonstrated to induce lipolysis, variations in its concentration were monitored, In the placebo group, ghrelin concentrations considerably increased. there were a trend toward a fall in the ghrelin in liraglutide group conclude that in the state of fasting, the patients who have inadequately controlled type I diabetes have ongoing ketogenesis and lipolysis, as it has been reflected in the progressive increase in the concentrations of β-hydroxybutyrate and FFA acetoacetate, administrating liraglutide results in inducing dramatic preventions of the increase in the acetoacetate and beta-hydroxybutyrate concentrations besides the reduction of the ghrelin, glucagon, and FFA concentrations (Nalla *et al*., 2020).

4.4.3. Obestatin

Statistical analysis of obestatin concentration in the table (4-12) showing there were insignificance increase ($P \geq 0.05$) in obestatin concentration of patient males when compared with controls males, the obestatin concentration is (110.072 and 99.694) nmol/mL, But in female's patients observed considerable rise in the obestatin concentration as a comparison to controls group ($P \leq 0.001$) the obestatin concentrations of patient's females and control is (113.675and 79.611) nmol/mL, Also in patients with TIDM observed considerable rise in the obestatin concentration as a comparison to controls group ($P \leq 0.001$) the obestatin concentrations of patients and controls is (112.378 and 87.645) nmol/mL, Finally, showed no significance difference ($P \geq 0.05$) in obestatin concentration between males to females for patients group as concentration (110.072 and 113.675) nmol/mL . Also found high significance increase ($P \leq 0.001$) in the obestatin concentrations of patient's females and males of control group (99.694 and 79.611) nmol/mL, .

Table 4-12: Concentration of Obestatin patients with TIDM and compare with control group

obestatin	Patients	Controls	P_{value}
	Mean ± SD nmol/mL	Mean ± SD nmol/mL	
Male	110.072±61.247	99.694±29.23	0.3467
Female	113.675±59.726	79.611±13.76	0.0001 **
Total	112.378±60.098	87.645±23.381	0.0002 **
P value	0.7258	0.0001 **	

** indicates the high significance difference ($p \leq 0.001$)

Serum obestatin levels of the T1DM patients in this work were found to be higher than those of the controls in table (4-12). A few researches carried out thus far support this idea. (Kolodziejski *et al.*, 2017) reported agreement findings, which are in agreement with recent results that found higher serum obestatin levels in both

types of diabetes. Obestatin levels were reported to increase in the patients who have the metabolic syndrome, obesity, T1DM, impaired glucose control, and Prader-Willi syndrome (associated with the obesity), while bariatric surgery-induced weight loss in the obese as well as the T2DM patients has shown to have no impact on the levels of the obestatin (Green & Grieve *et al.*,2018). Current findings are supported by the role of obestatin, Additionally, it seems to have effects on pancreatic β-cell, most notably increasing the beta-cell mass and activating genes linked to β-cell regeneration and insulin production, inhibiting glucose-induced insulin secretion and preventing lipolysis, acting similarly to insulin by lowering insulin resistance and adipocyte, inflammation that takes place in tissue with a high rate of metabolism, and acting similarly to insulin by reducing adipocyte, inflammation that occurs in tissue with high rate of metabolism, since those are (Balaky & Kakey, 2021). This results may be due to, It's actions on pancreatic beta-cells, most notably raising beta-cell mass and activating genes that are linked to the insulin synthesis and upregulating the regeneration (Balaky & Kakey, 2021).

4.4.4. Cholecystokinin (CCK)

The result of table (4-13) found no any significant different in CCK concentration of male's patients when compared to the control group. while there was high significant increase ($P \leq 0.05$) in the CCK concentration in female's patients with TIDM when compared with the control group, the concentration of CCK for T1DM patients and the control (21.002 and 13.071) nmol/mL, also there were a significant increase ($P \leq 0.05$) in the CCK concentration of patients with TIDM compared to control group (20.693 and15.739) nmol/mL .

Table 4-13: Concentration of CCK patients with TIDM and compare with control group

CCK	Patients	Controls	P_{value}
	Mean ± SD nmol/mL	Mean ± SD nmol/mL	
Male	20.143±12.645	19.741±19.422	0.9055
Female	21.002±12.304	13.071±2.686	0.0001 **
Total	20.693±12.392	15.739±12.784	0.0034 **
P value	0.6851	0.0145 *	

* indicates the significance difference (p≤0.05) ** indicates the high significance difference (p≤0.001)

Table (4-13) showed an increase in CCK levels of the females in patients group compared to controls, while there aren't any significant differences in the cck concentrations according to gender in the group of patients. which has a good impact on obesity and glucose metabolism, and stimulation of insulin secretion (Pathak *et al*., 2018). Whether obesity and T2DM effect CCK secretion is controversial because of the lack of a good experimental model to study I cell secreting CCK, in some studies serum CCK level were decrease in obese subject, but not in other (Filippello *et al.*, 2022). Cholecystokinin (CCK) is a hormone that is responsible for stimulation of fat and protein digestion, its presence leads to releasing bile and digestive enzymes from gallbladder and pancreas, , which slows the gastric emptying and acts as well as suppressant of hunger, Other CCK release factors, like the parasympathetic impulses and intra-luminal releasing factors could be responsible for the increase in the CCK levels after the bariatric surgery (Dimitriadis *et al.*, 2017). in the diabetic population, the symptoms that were reported included nausea, postprandial fullness, vomiting, bloating, early satiety, abdominal pain, belching, weight loss and heartburn, those disturbances may lead to symptoms that are consistent with the functional dyspepsia and gastroparesis, in addition to the delayed gastric emptying (Chedid *et al*., 2019)**.** GI functions are two of the main causes of blood glucose rises following meals: i) Gastric emptying that controls rate at which the glucose appears in small intestine and typically in the blood, ii) release of the

incretin hormones, GI hormones stimulating the secretion of the insulin (Steinert *et al*., 2017).

4.5. lipid profile (TG, TC, HDL, LDL and VLDL)

From the observation of the statistical analysis for the results of TG in table (4-14) there were found a significant increase ($P \leq 0.05$) in the concentration of TG for male's patients with TIDM when compared with controls group, the concentration of TG for patients and controls is (176.259 and 108.166) mg/dl, while observed high significant increase ($P \leq 0.001$) in the concentration of TG for female's patients with TIDM when compared with controls group, the concentration of TG for patients and controls is (170.666 and 94.777) mg/dl . also there were high significant increase ($P \leq 0.001$) in the concentration of TG for patients when compared to the controls, the concentrations for patients and controls is (172.68 and 100.133) mg/dl, lastly showed no significant difference in the concentration of TG in patients group and controls group according Separately.

The results of the table (4-14) showed there were high significant increase ($P \leq 0.001$) in the concentrations of TC for all results of T1DM patients groups compared to controls groups, the concentrations of TC in male patients compared to controls is (190.592 and 143) mg/dl, and female patients compared to control is (196.166 and 151.111) mg/dl. And the concentrations of TC in patients with T1DM compared to controls is (194.16 and 147.866) mg/dl, while in the same table observed no significant difference in the concentrations of TC ($p \geq 0.05$) in both groups patients and controls based on gender.

As indicated in table (4-14) there were insignificant decrease ($P \geq 0.05$) in the concentration of HDL in T1DM patients for males compared to controls, the mean of HDL in T1DM patients for males compared to controls is (40.259 and 42.166)

mg/dl. There was high significant decrease ($P \leq 0.001$) in the concentration of HDL for female's patients with T1DM compared to controls, the mean of HDL is (38.062 and 46.666) mg/dl. And high significant decrease ($P \leq 0.001$) in the concentration of HDL in patients with T1DM compared to controls groups, the HDL concentration is (38.853 and 44.866) mg/dl. Finally showed there were insignificant increase ($P \geq 0.05$) in the concentration of HDL of males compared to females in patients group, the mean of HDL (40.259 and 38.062) mg/dl. while showed high significant decrease ($P \leq 0.001$) in the concentration of HDL of males compared to females in control group, the mean of HDL is (42.166 and 46.666) mg/dl,

Results of table (4-14) showed high significant increase ($P \leq 0.001$) in LDL level in all groups of patients with T1DM as comparison to controls groups, the concentration of LDL for males in patients and controls is (126.333 and 67.833) mg/dl. And the concentration of LDL for females in patients and controls is (134.791 and 76.888) mg/dl. And the concentration of LDL for patients with T1DM as comparison to controls is (131.746 and 73.266) mg/dl. whereas in the same table observed no significant differences ($P \geq 0.05$) in the level of LDL in both groups of patients and controls according to gender.

The findings of table (4-14) showed there were significant increase ($P \leq 0.05$) in the concentration of VLDL in T1DM patients of males compared to controls, the mean of VLDL for males T1DM patients and controls is (34.962 and 24.166) mg/dl. Also there were high significant increase ($P \leq 0.001$) in concentration of VLDL for females with T1DM were compared to controls, the concentration of VLDL is (36 and 19.888) mg/dl. And observed there were high significant increase ($P \leq 0.001$) in concentration of VLDL of patients T1DM compared to controls, the concentration of VLDL is (35.626 and 21.6) mg/dl. While showed no significant difference ($P \geq 0.05$) in concentration of VLDL between males and females of patient groups, but

there was significant increase ($P \geq 0.05$) in concentration of VLDL in controls groups, the concentration of VLDL (24.166 and 19.888) mg/dl.

Table 4-14: Concentrations of lipid profile in patients with TIDM and compare with control groups

TG	Patients	Controls	P_{value}
	Mean ± SD mg/dl	Mean ± SD mg/dl	
Male	176.259±144.135	108.166±44.487	0.0074 *
Female	170.666±137.094	94.777±50.232	0.0001 **
Total	172.68±139.683	100.133±48.212	0.0001 **
P value	0.8142	0.1985	
TC	Patients	Controls	P_{value}
	Mean ± SD mg/dl	Mean ± SD mg/dl	
Male	190.592±54.389	143±32.884	0.0001 **
Female	196.166±51.136	151.111±18.838	0.0001 **
Total	194.16±52.393	147.866±25.545	0.0001 **
P value	0.5321	0.1409	
HDL	Patients	Controls	P_{value}
	Mean ± SD mg/dl	Mean ± SD mg/dl	
Male	40.259±8.505	42.166±4.116	0.2148
Female	38.062±7.273	46.666±4.093	0.0001 **
Total	38.853±7.809	44.866±4.642	0.0001 **
P_{value}	0.0972	0.0001 **	
VLDL	Patients	Controls	P_{value}
	Mean ± SD mg/dl	Mean ± SD mg/dl	
Male	34.962±20.164	24.166±10.131	0.0039 *
Female	36±22.981	19.888±8.288	0.0001 **
Total	35.626±22.019	21.6±9.258	0.0001 **
P_{value}	0.7820	0.0309 *	

* indicates significant difference ($p \leq 0.05$) ** indicates high significance difference ($p \leq 0.001$)

Triglyceride (TG)

The results in table (4-14) showed there were an increase in triglyceride concentrations in patients with the T1DM compared with healthy subjects, these results agreement with studies which indicated increase level of TG. agreement with

other study which say, high triglyceride levels are becoming more common due to the deterioration of life-style factors, diabetes, and obesity (Laufs *et al.*, 2020). In type I diabetes research, which had reported higher levels of triglyceride and LDL in the men who have type 1 diabetes compared with the women, this may not be agreement with our study, perhaps due to the difference in the nature and quality of nutrition in Iran(Shah *et al.*, 2018). Same findings were reported by *(*Kolodziejski *et al*., 2017) they clarified an increase the levels of TG and found that they were high in the all types of DM compared to the healthy group. Blood TG levels that are lower than 150mg/dL are considered to be normal, whereas the levels that are from 150mg/dL to 199mg/dL are borderline high and 200mg/dL to 499mg/dL are high, Due to aberrant lipoprotein metabolism, the high TGs level over 500mg/dL) in serum is utilized as bio-marker for the diabetes, Alzheimer's disease (AD), pancreatitis, and cardiovascular disease (CVD) (Dimache *et al*., 2021). However, in some research, it has been found that the T1DM had only a significant increase in the triglyceride and lower levels of the HDL, which showed typical dyslipidemia of the patients who have the metabolic syndrome (Fellinger *et al.,* 2019). Abd El Dayem have carried out a study on the type I diabetic patients and had shown that there has been a prevalence of the dyslipidemia in the patients where the triglycerides, LDL cholesterol and total cholesterol have been considerably higher whereas the HDL cholesterol has been reduced in comparison to the controls, which is why, should evaluate lipids soon after the diagnosis in the type I diabetic children who are older than 12 years, and it has to be repeated every 5 years in the case where normal results were obtained (Abd El Dayem *et al*., 2017).

Total Cholesterol (TC)

According to current finding in table (4-14) significantly increase in TC concentration in patients group with TIDM while no significant difference according

to gender, Despite the significant differences between the patients and the control group, we notice that the cholesterol values are within the normal level. We may attribute the reasons for this to the presence of a large number of children and adolescents in the study. The diabetes patients had higher diastolic blood pressure, total cholesterol, HbA1c, apoB, LDL cholesterol, body weight, apoA-I, BMI and waist circumference in comparison to the controls, both at the baseline and at five-year follow-ups (Heier *et al.*, 2017). Even though diurnal profiles of the cholesterol and triglycerides had shown subtle differences between controls and patients with a slight elevation in the concentration levels in T1DM group, the overall ranges of concentration have been rather similar in the two groups (Trefz *et al*., 2019). cholesterol levels in the diabetic patients has been higher in comparison with its levels in the healthy individuals (Alghazeer *et al*., 2018). Despite the slight increase, there were no significant differences in total cholesterol, total HDL cholesterol and triglycerides between T1DM and healthy participants (Gourgari *et al*., 2018).

High- Density Lipoprotein (HDL)

The results of table (4-14) showed a significant decrease in HDL concentrations in T1DM patients compared to the controls. low HDL-C much more often happen in the patients who have the metabolic syndrome or DM, chronic renal insufficiency, coronary disease, cardiovascular risk factors and disorders, where the HDL function has been impaired (März *et al.*, 2017). Low HDL-C is substantially more common in patients who have metabolic syndrome or DM than it is in those with genetically defined problems in HDL metabolism (März *et al.*, 2017). Another study disagrees with us had shown that T1DM patients usually have normal or even increased plasma HDL cholesterol concentration levels, None-the-less, the composition of HDL protein may be altered without changing the cholesterol content, HDL proteome alteration could lead to dysfunctional HDL particles with

decreased capability for protecting from CVD (Gourgari *et al.*, 2019). There have not been any significant differences in the total cholesterol, triglycerides and total HDL cholesterol between the T1DM and healthy participants (Gourgari *et al.*, 2018). It was established that the low HDL-C concentration, an independent CVD risk marker, coincides with the reduction of the protective capacity from the oxidative stress. None-the-less, conflicting results were reported on the low HDL-C levels' prevalence in the T1DM (Ganjali *et al*., 2017). Which is why, the low concentration of the HDL that was observed in the diabetic patients in comparison with the non-diabetic persons has been considered as a primary cause of the increased CVD risks (Srivastava, 2018).

Low-Density Lipoprotein (LDL)

The results of LDL as shown in the table (4-14) had high significant increase in level of LDL for diabetic patients compared with the healthy individuals and there were also clarified that there have not been significant differences between males and females in the group of patients, these results are similar and deals with some previous research. LDL cholesterol has been found increased significantly in comparison to healthy controls (Alghazeer e*t al.,* 2018). Total LDL-c and cholesterol have been significantly higher while the HDL cholesterol has been decreased in comparison to the controls, indicating increased risk of CVD.(Abd El Dayem *et al*., 2017). Another study indicated high concentrations of LDL in diabetic patients (Heier et al., 2017). In another study that has been conducted by *(*Wędrychowicz *et al*., 2019) to identify the variation in LDL–c concentrations in the diabetic patients compared to controls, this study had shown a non-significant increase. being higher in the T1DM compared with the controls (Gourgari *et al*., 2018).

Very Low-Density Lipoprotein (VLDL)

The present study clarified that the VLDL was significantly rise in the diabetic patients. Some study demonstrated the triglycerides and total cholesterol levels have been high in the diabetics, there were no significant differences in total HDLp, large HDLp and total, medium and large VLDLp, (Gourgari *et al.,* 2018). Results of some study had shown significant differences in the HDL, TC and LDL/VLDL levels between the patients and the healthy control group ($p < 0.0001$) (Bo *et al.*, 2019). Another study it was also clarified there have not been any significant differences in VLDL concentration between tow group diabetic patients and healthy *(*Huang *et al*., 2018).

4.6. The Correlations between parameters of the control group

Table (4-15) showed there were significant correlation at ($p \leq 0.01$) and ($p \leq 0.05$) between age with height, weight, BMI, HbA1c, glucose and cholesterol as it was positive correlation (0.661, 0.698, 0.692, 0.56, 0.768 and 0.678) . In the same time there were significant correlation at ($p \leq 0.01$) and ($p \leq 0.05$) between length and both weight, BMI and glucose and between weight with BMI, HbA1c, glucose, cholesterol, TG and LDL as it was positive correlation too (0.888, 0.796, 0.614, 0.981, 0.625, 0.791, 0.644, 0.552 and 0.501). And significant correlation at ($p \leq 0.05$) between HbA1c with glucose and cholesterol and between glucose and cholesterol as it was positive correlation too (0.615, 0.614 and 0.645). Also significant correlation at ($p \leq 0.01$) and ($p \leq 0.05$) between TG with LDL, VLDL, CCK and leptin as it was positive correlation too (0.641, 0.855, 0.513 and 0.56). Also there were significant correlation at ($p \leq 0.01$) between LDL with VLDL and CCK as it was positive correlation (0.732 and 0.537). also significant correlation at ($p \leq 0.05$) between VLDL with CCK, leptin and ghrelin as it was positive correlation too (0.684, 0.67, and 0.589). significant correlation at ($p \leq 0.01$) also can be seen between CCK with leptin, ghrelin and obestatin as it was positive correlation (0.893, 0.762 and 0.708). Significant correlation at ($p \leq 0.01$) and ($p \leq 0.05$) also can be seen between leptin with ghrelin and obestatin as it was positive correlation (0.814 and 0.575). At the same time there were significant correlation at ($p \leq 0.01$) between ghrelin and obestatin as it was positive correlation (0.748).

Table 4-15: The correlation between parameters in control group.

Control	Age	Length	Weight	BMI	HbA1c	Glucose	CHO	TG	HDL	LDL	VLDL	CCK	Leptin	Ghrelin	Obestatin	MDA	GSH
Age	1	0.661*	0.698*	0.692*	0.560*	0.768**	0.678*	0.203	0.145	0.268	0.234	-0.052	-0.018	-0.256	-0.082	0.044	0.259
Length		1	0.888**	0.796**	0.361	0.614*	0.470	0.454	-0.162	0.471	0.418	0.208	0.096	-0.008	0.196	0.229	0.228
Weight			1	0.981**	0.625*	0.791**	0.644*	0.552*	0.010	0.501*	0.462	0.092	0.031	-0.140	0.002	0.048	0.205
BMI				l	0.676*	0.827**	0.676*	0.534*	0.109	0.489	0.424	-0.007	-0.036	-0.223	-0.115	-0.037	0.183
HbA1c					1	0.615*	0.614*	0.079	0.164	0.096	0.029	-0.302	-0.372	-0.494	-0.267	-0.252	0.274
Glucose						1	0.645*	0.421	0.246	0.274	0.303	-0.124	-0.028	-0.135	-0.078	0.085	0.02
CHO							1	0.404	0.169	0.609*	0.497	0.136	0.046	-0.047	0.047	-0.119	-0.01
TG								1	-0.189	0.641*	0.855**	0.513*	0.560*	0.363	0.168	0.105	0.257
HDL									1	0.0831	-0.314	-0.122	-0.052	-0.282	-0.307	0.296	-0.21
LDL										1	0.732**	0.537*	0.445	0.277	0.393	-0.004	-0.007
VLDL											1	0.684*	0.67*	0.589*	0.453	-0.072	0.047
CCK												1	0.893**	0.762**	0.708**	0.370	-0.075
Leptin													1	0.814**	0.575*	0.398	-0.155
Ghrelin														1	0.748**	0.202	-0.483
Obestatin															1	0.176	-0.424
MDA																1	-0.028
GSH																	l

* the correlation has been significant at 0.05 level (2- tailed) ** the correlation has been significant at 0.01 level (2-tailed)

4.7. The Correlations between parameters of the TIDM patients group

Table (4-16) showed there were significant correlation at ($p \leq 0.01$) and ($p \leq 0.05$) between age with duration, height, weight and BMI as it was positive correlation (0.661, 0.581, 0.616 and 0.547) . In the same time there were significant correlation at ($p \leq 0.01$) and ($p \leq 0.05$) between height and both weight and BMI and between weight and BMI as it was positive correlation too (0.722, 0.554 and 0.966) . And significant correlation at ($p \leq 0.01$) and ($p \leq 0.05$) between cholesterol and both triglyceride and LDL as it was positive correlation too (0.603 and 0.517). Also there were significant correlation at ($p \leq 0.01$) between TG and VLDL as it was positive correlation (0.867). significant correlation at ($p \leq 0.01$) also can be seen between CCK with leptin, Ghrelin, Obestatin and MDA as it was positive correlation (0.802, 0.829, 0.898 and 0.746). Significant correlation at ($p \leq 0.01$) also can be seen between leptin with ghrelin, obestatin and MDA as it was positive correlation (0.791, 0.791 and 0.661). Also there were significant correlation at ($p \leq 0.01$) between ghrelin and both obestatin and MDA as it was positive correlation (0.826 and 0.614). At the same time there were significant correlation at ($p \leq 0.01$) between obestatin and MDA as it was positive correlation (0.708).

Table 4-16: The correlation between parameters in T1DM patients group.

Patients	Age	Duration	Length	Weight	BMI	HbA1c	Glucose	CHO	TG	HDL	LDL	VLDL	CCK	Leptin	ghrelin	Obestatin	MDA	GSH
Age	1	0.661*	0.581*	0.616*	0.547*	-0.287	-0.005	0.064	0.05	0.062	0.1	0.184	-0.115	-0.094	-0.332	-0.074	-0.108	0.334
Duration		1	0.266	0.369	0.323	-0.058	-0.032	0.101	0.142	-0.095	0.019	0.163	-0.011	-0.012	-0.118	-0.002	-0.126	0.172
Length			1	0.722**	0.554*	-0.29	0.177	0.104	-0.029	-0.006	0.097	-0.031	-0.263	-0.227	-0.277	-0.196	-0.245	0.232
Weight				1	0.966**	-0.450	-0.015	-0.028	-0.002	-0.045	0.115	0.121	-0.197	-0.208	-0.221	-0.091	-0.281	0.251
BMI					1	-0.461	-0.059	-0.061	0.002	-0.045	0.140	0.171	-0.179	-0.188	-0.189	-0.073	-0.254	0.274
HbA1c						1	0.271	0.136	0.189	0.007	0.134	0.102	0.062	0.106	0.065	0.019	-0.012	-0.176
Glucose							1	-0.031	-0.091	0.084	-0.075	-0.086	-0.071	-0.010	-0.054	0.016	-0.109	0.057
CHO								1	0.603*	0.285	0.517*	0.396	-0.159	-0.114	-0.163	-0.161	-0.175	0.127
TG									1	0.027	0.421	0.867**	-0.023	-0.031	-0.026	-0.064	-0.122	0.002
HDL										1	0.416	0.101	-0.105	-0.001	-0.121	-0.065	-0.014	0.215
LDL											1	0.467	-0.172	-0.064	-0.131	-0.201	-0.235	0.112
VLDL												1	-0.055	-0.091	-0.096	-0.1	-0.151	0.061
CCK													1	0.802**	0.829**	0.898**	0.746**	-0.208
Leptin														1	0.791**	0.791**	0.661*	-0.038
Ghrelin															1	0.826**	0.614*	-0.212
Obestatin																1	0.708**	-0.074
MDA																	1	-0.114
GSH																		1

* correlation has been significant at 0.05 level (2- tailed) ** correlation has been significant at 0.01 level (2-tailed)

In the present study, we reported that there was a statistically significant positive correlation between the age with BMI and between lipid profile in the diabetic and the non-diabetic group. Even though the treatment with insulin results in improving the metabolism of the glucose in the T1DM, diabetes-associated disease burden is increased with the time for development of the chronic complications that are related to long-term HbA1c levels and duration of diabetes in the patients who have poor metabolic control, Type I diabetes patients show impaired lipid profiles that could be related to developing late diabetic complications (Ganjali *et al.*, 2017).

At the same time, no association between lipid peroxidation (MDA) and antioxidant enzyme that evaluated, was nonenzymatic (GSH). In T1DM, there was no link between the lipid profile parameters (cholesterol, TAG, HDL, and LDL) and the peroxidation parameters like MDA, the antioxidants GSH, or the HbA1c, these results are supported by several previous studies conducted by several researchers, like Alghobashy which cleared no relation between (MDA) and (GSH) In T1DM patients, and no link between the lipid profile parameters and the antioxidants or HbA1c in the type 1 diabetes (Alghobashy *et al.*, 2018). Other which Revealed links between glycemic variability and erythrocyte membrane stability and lipid peroxidation (MDA), highlighting its role in mechanisms relating to emergence of chronic problems in T1DM patients (Abdel-Moneim *et al.*, 2020).

Additionally, a strong positive correlation between hormones (Leptin, Ghrelin, CCK, Obestatin) that evaluated in the present study, as well as between all of them with lipid peroxidation (MDA) in the T1DM group. agreement with other recent study which found that the lack of the correlation of the levels of serum ghrelin with the HbA1c and the levels of the ghrelin aren't affected by the BMI (Özcan *et al.*, 2021). adding more evidences to idea that the leptin and CCK have

synergistic actions on the suppression of the appetite, the CCK has been recognized to improve the permeability of the blood–brain barrier to the leptin and encourage the release of the leptin from the adipose tissues, Similarly, in reciprocal manner, it's believed that the leptin can result in stimulating the secretion of the CCK (Pathak *et al.,* 2018). There's also a suggestion that the activation of CCK1 receptor results in stimulating the secretion of adipocyte-derived hormone, leptin that helps as well in controlling the intake of energy (Pathak *et al.,* 2018). In addition to other prominent physiological CCK actions, CCK, along with the secretin and gastrin, have been largely considered as a family of related peptides with the purpose of digestion regulation with the positive impacts on the obesity and glucose metabolism and stimulations of the secretion of insulin (Navarro-Guillén *et al.,* 2017). Insulin leads to stimulating release of the preformed and newly synthesized leptin by the adipocytes via its signaling cascade (Marques-Oliveira *et al.,* 2018). Leptin is an adipocyte-derived hormone that stimulates in regulating energy intake, and there is evidence that its secretion is stimulated by CCK-1 receptor activation (Pathak *et al.*, 2018). Insulin, leptin, and ghrelin are all known as regulators of appetite and energy balance, Insulin and ghrelin also both show reciprocal correlations during the course of the day, with the levels of the insulin being high when ghrelin levels are low and vice versa, It is the antagonist of the hormone leptin, which is produced by adipose tissue and induces a feeling of satiety when it is present in high levels, Single intravenous administration of the ghrelin results in increasing the levels of the plasma glucose followed by drop in the levels of the fasting insulin in lean (Warchoł *et al.*, 2018).

Inversely, other study which has been done by other researcher, which cleared a strong positive connection between HbA1c and MDA was found, indicating that poor metabolic control is linked to greater MDA expression (de Souza Bastos *et al.*,

2016). however there has not been any statistically significant relationship between BMI, age, Cu, and insulin dose (Alghobashy *et al.*, 2018). The levels of the Leptin have shown significant positive association with the circumference of the hip and BMI and negative correlations with the HbA1c in the overweight diabetics, did not find any significant correlations between the age and the serum LDL-C of diabetic patients (Abd El Dayem *et al.*, 2017). Obestatin and insulin were found to be negatively correlated, Total ghrelin, active ghrelin, and serum obestatin did not correlate with one another (Kolodziejski *et al.*, 2017). HbA1c % had statistically significant positive correlation with illness duration, The dose of the insulin had shown statistically significant positive correlation with the age (Alghobashy *et al.*, 2018).

Conclusions and Recommendations

Conclusions

1. There is an association between diabetes and age, as it was observed that most of the patients with T1DM were children and adolescents whose ages ranged between (5-25).
2. Most of those diagnosed with T1DM are female, as the number of females that have participated in the study has been twice the number of males.
3. Increase in the MDA concentration which mean the rate of oxidative stress is high, MDA is more commonly used as a biomarker of oxidative stress
4. Decrease in the antioxidant concentration (GSH) in both patients T1DM.
5. It was also noted that there was an increase in the level of hormones Leptin, Ghrelin, Obestatin and CCK, that regulate the metabolism, which may indicate the presence of metabolism disorder in both patients T1DM.
6. People with type 1 diabetes have an uncontrolled lipid profile, where a marked increase in TC, TG, LDL and VLDL levels and a significant reduction in the HDL levels in the patients compared with healthy group.

Recommendations

1. Studying the effects of different treatments on the levels of the bio parameters in patients with T1DM
2. Study the effect of diet on diabetic patients
3. Comparison of study parameters with diabetic patients who had controlled glucose levels and those who had uncontrolled glucose level

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Appendicaes

Apendix 1:

Questioner for participants

Name: address: height:

Age: duration: weight:

Phone Number:

Serial	Content	
1.	Work	
2.	Simple date	
3.	Family history	
4.	Other dieses	
5.	Any treatment	
6.	BMI	

Serial	Content	
1.	ABO group	
2.	HbA1C	
3.	Fasting blood sugar	

Serial	Enzymes	
1.	GSH	
2.	MDH	

Serial	Hormones	
1.	Leptin	
2.	Ghrelin	
3.	Obestabin	
4.	Cholecytokinine	

Serial	Lipid profile	
4.	Total cholesterol	
5.	LDL	
6.	HDL	
7.	Triglyceride	

الخلاصة

داء السكري هو مجموعة من الأمراض الأيضية التي تتميز بارتفاع سكر الدم المزمن الناتج عن عيوب في إفراز الأنسولين، أو عمل الأنسولين، أو كليهما ويمكن تصنيفه إلى داء السكري من النوع الأول (تدمير خلايا بيتا في غدة البنكرياس بواسطة الخلايا المناعية الذاتية، مما يسبب نقصًا مطلقًا في الأنسولين)، داء السكري من النوع الثاني (مزيج من انخفاض إفراز الأنسولين وانخفاض حساسية الأنسولين الى لمستقبلاته (أي مقاومة الأنسولين)، وسكري الحمل بالإضافة الى أنواع محددة أخرى من داء السكري.

اجريت الدراسة خلال ستة أشهر من تشرين الثاني/2021 الى نيسان/2022 في مركز الحسن للسكري والغدد الصم في مدينة الامام الحسين الطبية بكربلاء. العدد الكلي للمشاركين هو (240) شخصًا يشمل (150) مريضًا مصابًا بداء السكري من النوع الأول، الذكور54 (36٪)، والإناث 96 (64٪) و (90) شخص اصحاء غير مصابين بالسكري، الذكور 36 (40٪)، الإناث 54 (60٪).

تم الحصول على عينة من الدم الوريدي حجمها خمسة مل من المرضى والأصحاء. بالإضافة إلى ذلك، تم أخذ بعض المعلومات من كل شخص والتي تشمل (العمر والجنس ومدة تاريخ المرض والطول والوزن). حيث تم اجراء مجموعة من الاختبارات بواسطة مجموعة من عدة الفحص والاجهزة لقياس فصيلة الدم ونسبة الجلوكوز والدهون، بالإضافة الى المعايير الاساسية في دراستنا التي تشمل بعض من الهرمونات ومضادات الاكسدة، التي تم قياسها بجهاز الايلايزا في مختبر النقاء ماعدا اختبار السكر التراكمي الذي اجري في مركز الحسن.

في هذه الدراسة لوحظ، انه اعلى معدل من المصابين بالنوع الأول من السكري كانوا ضمن الفئات العمرية التي تتراوح أعمارهم بين (17-12) 48٪ و (23-18) 16٪ وهذا ما يؤكد أن النسبة العالية من المرضى هم من الأطفال والمراهقين. كما لوحظ أن اغلب المرضى الذين تم تسجيلهم في هذه الدراسة وتم تشخيص إصابتهم بالسكري هم من الإناث وكانت نسبتهم (64٪) بينما كانت نسبة الذكور (36٪) وهذه النتيجة أظهرت وجود علاقة بين الجنس ومرض السكري من النوع الأول.

أظهرت الدراسة وجود ارتباط معنوي بين الوزن والطول ومؤشر كتلة الجسم والجنس اما فيما يتعلق بفصيلة الدم لاحظت الدراسة الحالية أن أعلى نسبة من المصابين بالسكري من النوع الأول كانت فصيلة دمهم O +

(48٪) تليها فصيلة الدم A + بنسبة (25.43٪) .بينما زادت تراكيز الجلوكوز والسكر التراكمي عند المرضى وخاصة الإناث مقارنة بالمجموعة الصحية.

لوحظ في معظم المرضى زيادة في تركيز إنزيم MDAوانخفاض في تركيز إنزيم GSH. أيضا زيادة في تركيز كل من الهرمونات (Leptin, Ghrelin, Obestatin and CCK)في المرضى من فئة الاناث مقارنة بالذكور عند مقارنتها بمجموعة الاصحاء. ولوحظ أيضًا زيادة كبيرة في إجمالي مستويات CHO و TGو LDLو VLDLوانخفاض كبير في مستويات HDL في المرضى مقارنةً مع مجموعة الاصحاء، كذلك ارتفاع الدهون في الإناث الأكثر من الذكور.

بينت هذه الدراسة عدة علاقات بين المتغيرات الفسيولوجية في مجموعة المرضى، حيث كانت هناك علاقة معنوية بين العمر ومدة المرض والطول والوزن ومؤشر كتلة الجسم، كذلك علاقة معنوية بين الكوليسترول والدهون الثلاثية وLDL، وعلاقة معنوية بين VLDL TG. بالإضافة إلى الارتباط المعنوي بين CCK مع اللبتين، Ghrelin، Obestatin وMDA، أيضًا هناك ارتباط إيجابي بين اللبتين وكلا من Gharlin Obestatinو MDA.

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تقييم بعض الهرمونات والدهون وتركيز المؤكسدات / مضادات الأكسدة في مرضى السكري من النوع الأول في محافظة كربلاء

رسالة مقدمة

الى مجلس كلية العلوم الطبية التطبيقية - جامعة كربلاء

وهي جزء من متطلبات نيل شهادة الماجستير في التحليلات المرضية

بواسطة

ريام داخل محسن

بكالوريوس تحليلات مرضية/ 2017 كلية العلوم الطبية التطبيقية – جامعة كربلاء

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