Repuplic of Iraq
Ministry of Higher Education
And Scientific Research
Al-Nahrain University
College of Science
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Effect of Anti diabetic Drugs on Glucose-6-phosphatase Activity in Patients with Type 2 Diabetes Mellitus

A Thesis

Submitted to the College of Science/Al-Nahrain University as a partial Fulfillment of the Requirements for the Degree of Master of Science in Chemistry.

By

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March 2016 **Jamadi alakar 1437**

﴿بسم الله الرحمن الرحيم﴾

وَعَلَّمَكَ مَا لَمْ تَكُنْ تَعْلَمُ وَكَانَ فَضْلُ اللَّهِ عَلَيْكَ عَظِيمًا

صدق اللَّهُ العظيم

سورة النساء ﴿ الآية ١١٣﴾

Dedication

To the person who always be there for me , with his love,

Tenderness and support… …

My Dearest

Father

To the fountain of unconditional love and sacrifice

,who I still drinking from her love, warm and Affection

… …

My lovely

Mother

To persons who always give me the hope and reason to go on in my live… …

My lovely brothers and sisters

To whom bright my life with joy and happiness... ...

My wonderful friends Afaf and Mays

Zainab Salih

Acknowledgments

Praise be to Allah for his great help, guidance and for the strength and encouragement which he gave me to complete this thesis.

I would like to express my gratitude to my supervisor ***Assistant professor Dr. Alaa H. Jawad*** *for their valuable guidance and encouragement in supervising the present work.*

Deep appreciation to ***(Assistant professor Dr. perry H. Saifullah)*** *for her help, great interest, kindness, supportive pieces of advice throughout this work.*

I would like to thank the staff of National Diabetes center Al-Mustansiriya University and the staff of biochemistry lab for their help and advice to collect the samples.

I would like to thank the staff of Biotechnology Research Center at Al-Nahrian University for provision the facilities to complete the important part of my practical work and analyzing the results.

I express my special thanks and respect to my father specialist surgeon ***Dr. Salih Hallab*** *To cooperate with me and provide advice during the work.*

My special thanks and sense of gratitude to my especial friend ***Mays waad*** *who supported me during my study.*

Zainab Salih

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Summary

Background:

Type 2 Diabetes mellitus is a disease characterized by chronically elevated blood glucose levels, even when fasting largely due to increased endogenous glucose production. In liver, glucose-6-phosphatase catalyses the terminal step of glycogenolysis and gluconeogenesis. The classical role of glucose-6-phosphatase in the liver is the production of glucose for release into the blood. This correlation between type 2 diabetes and glucose-6-phosphatase makes this enzyme an appealing drug target for control of blood glucose levels as its inhibition would directly prevent the release of free glucose into the blood stream. Aims of the study were to assess the effects of anti-diabetic drugs(metformin and metformin plus glibenclamide) on Glucose-6-phosphatase activity, serum leptin concentration, Fasting plasma glucose (FPG) , Glycated hemoglobin (HbA1c%) and lipid profile in type 2 diabetic patients and to study the factors that affect the binding between Glucose-6-Phosphatase and biotin antibody in all diabetic patients and control.

Subjects, Materials and Methods:

This study includes (84) subjects, their age ranged from (40 to 54) years. (20) subjects were healthy chosen as control group and (64) patients with type 2 diabetes mellitus were divided into three groups according to their type of anti diabetic therapy : (23) newly diagnosed group without therapy (Group1) , (20) with metformin therapy (Group2) and (21) with metformin plus glibenclamide therapies(Group3) . In the first part of the study Glucose- 6 -Phosphatase (G-6-Pase) activity and Leptin hormone level were quantitatively determined in patients and healthy subjects by Enzyme-Linked Immuno sorbent Assay [ELISA] test.

In addition to that, the duration of disease, duration of taking treatment, fasting plasma glucose (FPG), Glycated hemoglobin (HbA1c%), body mass index (BMI), and lipid profile were identified in the patients. In the second part we study the characteristics of the binding of biotin antibody with G-6-Pase in the sera of the healthy subjects and patients. Different factors affecting this binding were studied such as concentration of antibody, concentration of antigen, temperature, and incubation time.

Results:

This study showed the following:

Part I:

- The levels of G-6-Pase activity and serum leptin hormone are found to be significantly higher in diabetic patients compared with healthy control group ($P<0.05$).
- The levels of G-6-Pase activity and serum leptin hormone were found to be significantly higher in newly diagnosed group when compared with metformin group, metformin plus glibenclamide group and control group($P<0.05$).
- A significant correlation ($P<0.05$) was found between G-6-Pase activity and FPG in all diabetics groups (newly diagnosed group, metformin group, metformin plus glibenclamide group) and a significant correlation between G-6-Pase activity and HbA1c % in newly diagnosed group and metformin group.

- No significant correlation ($P>0.05$) was found between the level of leptin and G-6-Pase activity and significant correlation ($P<0.05$) was found between the level of leptin and BMI in all diabetic groups.

Part II:

The binding studies of biotin antibody with antigen by using ELISA Test revealed that the optimum factors that affect the binding are: Concentration of biotin, Concentration of G-6-Pase enzyme, Temperature and Incubation time

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Abbreviations

Ab	Antibody
ADA	American Diabetes Association
Ag	Antigen
Ag-Ab	Antibody-Antigen
DCCT	Diabetes Complications and Control Trial
DKA	diabetic ketoacidosis
DM	Diabetes Mellitus
EGP	Endogenous glucose production
ELISA	Enzyme Linked Immunoabsorbent Assay
ER	Endoplasmic reticulum membrane
FPG	Fasting Plasma Glucose
G6P	Glucose-6-phosphate
G-6-Pase	Glucose- 6- phosphatase
GDM	Gestational Diabetes Mellitus
GHb	Glycated Hemoglobin
Glib.	Glibenclamide
GP	Glycogen phosphorylase
GSD	Glycogen storage disease
GTT	Glucose tolerance test
HbA1c	Hemoglobin Adult $_1$ glycated
HCC	Hyperosmolar Hyperglycemic State
HDL-C	High Density Lipoprotein Cholesterol
HGP	Hepatic glucose production
HHS	Hyperosmolar Hyperglycemic State
IDDM	Insulin-dependent diabetes mellitus
LA	Lactic acidosis
LDL-C	Low density Lipoprotein Cholesterol
LpL	lipoprotein lipase
MT.	Metformin
NIDDM	Non-insulin dependent diabetes mellitus
PEP	phosphoenolpyruvate
PEPCK	phosphoenolpyruvate carboxykinase
T2DM	Type 2 Diabetes Mellitus
TC	Total Cholesterol
TG	Triglyceride
TZDs	Thiazolidinediones
VLDL-C	Very Low Density Lipoprotein Cholesterol
WHO	World Health Organization

Chapter one

Introduction and Literature Review

1.1 Diabetes mellitus

1.1.1 Definition:-

Diabetes mellitus (DM) has been defined as a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both [(1)]. Insulin is a hormone produced in pancreas enable body cells to absorb glucose to turn into energy if the body cell do not absorb the glucose it will accumulates in its blood "hyperglycemia" The chronic hyperglycemia leading to various potential complication [(2)] . The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels [(1)].

1.1.2 The Classification of Diabetes Mellitus:

The new classification of DM has been proposed by American Diabetes Association (ADA) and World Health Organization (WHO). It comprises four etiological types: Type 1 diabetes (due to b-cell destruction , usually leading to absolute insulin deficiency),Type 2 diabetes (due to a progressive insulin secretary defect on the background of insulin resistance), Gestational diabetes mellitus (GDM) (diabetes diagnosed in the second or third trimester of pregnancy that is not clearly overt diabetes), Other specific types of diabetes due to other causes, e.g., monogenic diabetes syndromes (such as neonatal diabetes and maturity-onset diabetes of the young [MODY]), diseases of the exocrine pancreas (such as cystic fibrosis), and drug- or chemical-induced diabetes (such as in the treatment of HIV/AIDS or after organ transplantation) [(3)].

1.1.2.1 Type 1 diabetes

It was previously called insulin-dependent diabetes mellitus (IDDM) or juvenile-onset diabetes. Type 1 diabetes develops when the body's immune system destroys pancreatic beta cells resulting in failure of insulin production. This form of diabetes usually strikes children and young adults, although disease onset can occur at any age. Type 1 diabetes accounts for 5-10% of all diagnosed cases of diabetes [(4)]. This type of diabetes usually presents acutely with hyperglycemic symptoms include polyuria, thirst, tiredness, weight loss and drowsiness usually denote impending ketoacidosis [(5)].

1.1.2.2 Type 2 diabetes

Named non-insulin dependent diabetes mellitus (NIDDM) or adult onset diabetes [(1)] It was account for 90 -95% of those with diabetes. Type 2 diabetes mellitus is a heterogeneous disease which is characterized by variable degrees of insulin resistance, impaired insulin secretion and increased glucose production. Insulin resistance occurs when the cells become less sensitive to the effects of insulin [(6)]. The defective responsiveness of body tissues to insulin almost involved the insulin receptor in cell membrane. In the early stage the predominant abnormality is reduced insulin sensitivity characterized by elevated levels of insulin in the blood. At this stage, hyperglycemia can be reversed by a variety measures and medications that improve insulin sensitivity or reduce glucose production by the liver. As the disease progresses, the impairment of insulin often becomes disease and remains undiagnosed for a long time [(7)].

1.1.2.3 Gestational Diabetes Mellitus (GDM)

Gestational Diabetes Mellitus has been defined as any degree of glucose in tolerance with onset or first recognition during pregnancy. The prevalence of Gestational Diabetes Mellitus (GDM) can range from 2 to 3 % of pregnancies. Most women revert to normal glucose tolerance post partum but have a substantial risk of 30 to 60 % of developing DM later in their life .The risk factor for GDM includes:

1. Age.
2. Ethnicity.
3. Pregnancy body mass index.
4. Family history.[8]

1.1.2.4 Other specific types of diabetes

These types could be due to other causes:-

a. Genetic defects of β-cells function (e.g. maturity- onset diabetes of the young (MODY).

b. Genetic defects in insulin action, disease of the exocrine pancreas (pancreatitis, trauma, neoplasia, fibrocalouspancreatopathy) .

c. Endocrinopathie(acromegaly, Cushing's syndrome, pheochromocytoma)

d. Drug or chemical induced (glucocorticoids)

e. Infections (congenital rubella) [9][10] .

1.1.3 Diagnosis of Diabetes Mellitus

The National Diabetes Data group and the world health organization have issued DM diagnostic criteria, which are based on the following principles:

a.HbA1c: Glycated hemoglobin is a blood test that provides information about a person's average levels of blood glucose over the past 3 months. The HbA1c is the test used for diabetes management and diabetes research **(11).** One advantage of using A1C measurement is the ease of testing because it does not require fasting. An A1C level of greater than 6.5 percent on two separate occasions is considered diagnostic of diabetes **(12).**

- ***Structure of Glycated hemoglobin (GHb or HbA1c) :-***

Glycated hemoglobin have a carbohydrate moiety (glucose or a derivative) attached to one of the globin chains.

Carbohydrate may be attached to the N-terminus amino acid residue (valine) of the ß or α globin chains. Linkage to the N-terminus of ß-chains is of most practical importance since this rise to the altered physical properties **(13).**

- ***Formation of HbA1c:-***

Glucose combines with the α –amino group of the valine residue at the N-terminus of ß globin chain to form an aldimine (Schiff base)·. During red blood cell circulation, some of the Schiff base is converted to form a stable ketoamine **(14).**

Glycated hemoglobin is formed in two steps by the non-enzymatic glycation of HbA1c. In the first step glucose can rapidly react with hemoglobin to form labile aldimine pre- A1c (Schiff base) · The reaction between the carbonyl group of glucose and the N-terminal valine of β-chain of hemoglobin is reversible and dissociation to native hemoglobin and glucose occults readily. In the second step is irreversible when a labile aldimine (Schiff base) undergo an amadori rearrangement to form Glycosylated hemoglobin (keto amine) the keto amine product is stable and cannot revert back to hemoglobin and glucose **(15) .**

b.FPG: Fasting plasma glucose (FPG) is commonly used to screen for disorders of glucose metabolism, mainly diabetes mellitus and it is a useful monitor for drug or dietary therapy in patients with diabetes mellitus .This test determines the blood glucose level that reflects glucose control only at the moment of the obtained sample [16,17].

c. GTT: Glucose tolerance test is also called GTT, is a way of examination of how your body handles glucose. Glucose is given as a drink called polycal, blood samples are taken immediately before and 2 hours after this drink. The levels of glucose in the blood samples are measured and the result allow the doctor to make judgment on how well your body is able to manage blood sugar levels [18].

d. R.P.G: Random blood glucose concentration > 11.1 mmol/L(200mg/dL): random is defined without regard to time since the last meal [19].

1.2 Risk factors and symptoms of type 2 diabetes

The most common risk factors for type 2 diabetes comprise obesity, poor diet, sedentary lifestyle, increased age; 21% of people over 60 years have diabetes with family history; diabetes tends to run in families [20,21] . Not everyone with type 2 diabetes has symptoms, particularly in the early stages of the disease. In fact, 5.7 million of the 23.6 million people with diabetes are unaware that they even have the disease of those, 90 to 95% are those with type 2 diabetes [22] . People with type 2 diabetes may not have symptoms for years or decades, but as the disease progresses and blood sugar levels rise, symptoms develop. People with type 2 diabetes may have the following signs and symptoms:

- Blurred sight
- Decreased sensation or numbness in the hands and feet
- Dry, itchy skin
- Frequent bladder and vaginal infections » frequent need to urinate
- Increased thirst and hunger
- Male impotence (erectile dysfunction)
- Slow healing of cuts or sores
- Tiredness [(7)].

1.3 Complications of type 2 diabetes mellitus

Complications of type 2 diabetes include acute and chronic complications acute metabolic complications of diabetes consist of diabetic ketoacidosis (DKA), Hyperosmolar Hyperglycemic State (HHS) [(23)] , lactic acidosis (LA), and hypoglycemia [(24)] . Diabetic Ketoacidosis (DKA) and Hyperosmolar Hyperglycemic State (HHS) caused by absolute or relative deffiency in endogenous insulin level with concomitant rise of counter regulatory hormones such as catecholamines, glucagon, growth hormone and cortisol [(25)] . Hypoglycemia results from the treatment of diabetes, either with oral agents or insulin. Although hypoglycemia may occur in conjunction with oral hypoglycemic therapy, it is more common in patients treated with insulin [(24)] .LA is caused by the buildup of lactic acid in the bloodstream and occurs when oxygen levels in the body drop (hypoxia). The occurrence of LA in type 2 diabetes is of great concern because the mortality rate of LA can be as high as 50% [(26)]. The chronic complications include cardiovascular disease, peripheral vascular disease, cerebrovascular disease, diabetic retinopathy and diabetic nephropathy. These complications are associated with

considerable morbidity, reduced quality of life, disability, premature mortality and high economic costs [27].

1.4 Etiology

Type 2 diabetes is caused by a combination of genetic factors related to impaired insulin secretion and insulin resistance and environmental factors such as obesity, overeating, lack of exercise, and stress, as well as aging. It is typically a multifactorial disease involving multiple genes and environmental factors to varying extents [28].

1.4.1 Roles of environmental factors

- **Life style**

Epidemiological studies of type 2 diabetes provide evidence that over eating especially when combined with obesity and under activity is associated with the development of type 2 diabetes. Type 2 diabetes risks can be reduced in many cases by making changes in diet and increasing physical activity. Diets that are very low in saturated fats reduce the risk of becoming insulin resistance and diabetic. A number of life style factors are known to be important to the development of type2 diabetes. Persons who have high levels of physical activity, a healthy diet, none smokers, and consume alcohol in moderation have an 82% lower rate of diabetes. And when a normal weight has been included the rate has been 89% lower [29,30,31].

- **Obesity**

Obesity is a medical condition in which excess body fat has accumulated to the extent that it may have an adverse effect on health, leading to reduced life expectancy and increased health problems. People are considered as obese when their Body mass index (BMI), a measurement which compares their weight and squared height, exceeds 30 kg/m2 [(32)] .Obesity increases the likelihood of various diseases, particularly heart disease and type 2 diabetes. It is most commonly caused by a combination of excessive food energy intake, lack of physical activity, and genetic susceptibility, although a few cases are caused primarily by genes, endocrine disorders, medications or psychiatric illness [(33)]. Eighty percentage of people with type 2 diabetes are overweight when diagnosed diabetes symptoms disappear in many of these obese patients when they lose weight [(34)].

- **Pregnancy:** Hormones produced during pregnancy block the effect of insulin [(35)].

- **Age:** Type 2 diabetes is principally a disease of the middle aged and elderly. It affects 10% of the population over age of 65 years and over 70% of all cases of diabetes occur after the age of 50 years [(36)].

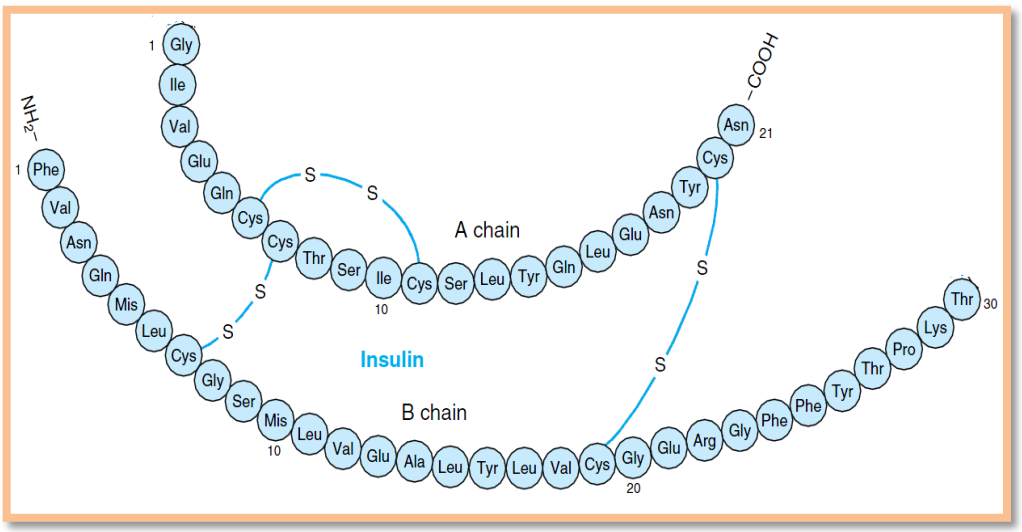
1.4.2 Genetic factor

Type2 diabetes is a complex polygenic disorder in which common genetic variants interact with environmental factors to unmask the disease. Genetic factors are known to play an important part in the development of Type2 diabetes [(37)]. Study of the occurrence of diabetes between monozygotic twins suggests a concordance of around 60% . The

marked differences in prevalence of type 2 diabetes between some ethic groups probably also represent differences in the genetic susceptibility **(38).** The common form of NIDDM is apparently inherited as a polygenic trait (a trait controlled by many genes) **(39).** with environmental factors which also playing a role. The risk to develop the disease increases strikingly, if there is a family history, especially among first degree relatives **(40).**

1.5 Insulin

Insulin is a polypeptide hormone produced by the β-cells of the Islets of Langerhans, clusters of cells that comprise about 1% of the mass of the pancreas. Insulin is one of the most important hormones coordinating the utilization of fuels example, synthesis by tissues. It is composed of 51 amino acids arranged in two polypeptide chains, chain (A) which contains 21 amino acids and chain (B) contains 30 amino acids which are linked together by disulfide bridges ,the insulin molecule also contains an intermolecular disulfide bridges between amino acid residues 6 and 11 of the A chain **(41)** Figure (1.1).



Figure(1.1) Human insulin **(41).**

Treatment of Type 2 diabetes

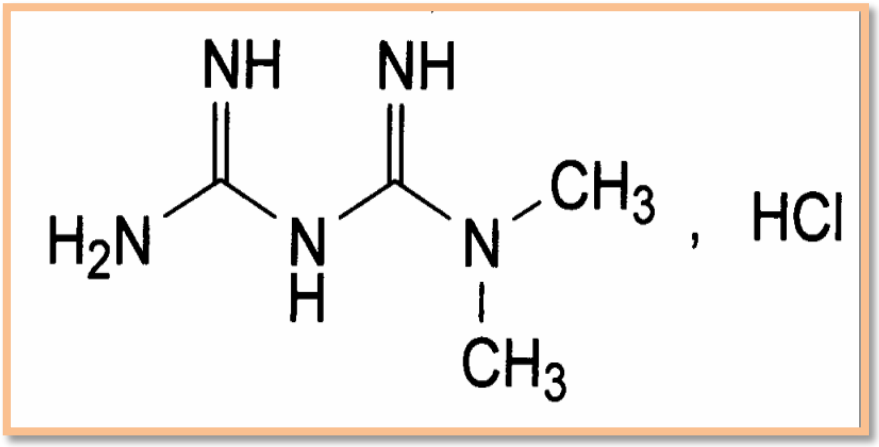
1.6 Oral hypoglycemic agents

Oral hypoglycemic agents have a very important clinical impact on treating type 2 diabetes. Many complications develop if the disease is not treated early enough and with the proper pharmacological agents. These complications include diabetic retinopathy, nephropathy, and neuropathy. Other risk factors must also be addressed in patients with type 2 diabetes. Old agents such as sulfonylureas, biguanides, TZDs, alpha-Glucosidase inhibitors, and short-acting insulin secretagogues, and combination therapies had a good impact on disease treatment. The clinical benefits of all pharmacological agents become more complete when accompanied with non-pharmacological treatments **(42).**Many oral hypoglycemic agents exist that have proven to be successful in lowering significant parameters in disease monitoring such as HbA1C, fasting blood glucose reduction and peak postprandial plasma glucose **(43).**

1.6.1 Metformin

Metformin (N, N-dimethylbiguanide) belongs to the biguanide class of anti-diabetic drugs chemically is 1,1-Dimethyl biguanide hydrochloride with a molecular formula of $C_4H_{12}Cl\ N_5$ (Figure 1.2) **(44).**

Metformin has been an important drug for treatment of type 2 diabetes (T2D) for decades. It is the most widely used oral antihyperglycemic agent and is currently recommended as first line therapy for all newly diagnosed T2D patients **(45).** Metformin has been used widely in the treatment of T2D for over 50 years and has been found to be safe and efficacious both as monotherapy and in combination with other oral anti-diabetic agents and insulin **(46).**



Figure(1.2) : Chemical structure of Metformin- HCl [(44)].

The work of Dr Jean Sterne, a French clinician and his colleagues led to the discovery of metformin as an oral antidiabetic agent in Paris . The first synthesis of metformin (dimethyl biguanide) is attributed to Werner and Bell from Trinity College, Dublin, Ireland, in 1922 , and was a basis for further experimental and clinical studies on the potential therapeutic application of biguanides, particularly metformin. The other two biguanide agents, phenformin and buformin, were soon withdrawn from widespread clinical use due to their toxicity, especially lactic acidosis. However, five decades were needed to promote metformin from a minor product to the 'gold standard' in the treatment of type 2 DM, with a wide safety profile [(47)].

1.6.1.1 Mechanisms of actions.

Metformin appears to have several, incompletely understood but complementing each other, modes of action [(48)].The main mechanisms include anorexiogenesis, reduction of intestinal carbohydrate absorption, inhibition of hepatic gluconeogenesis, as well as increased glucose uptake by peripheral tissues [(49)].

Metformin exerts its glucose-lowering effect primarily by decreasing hepatic glucose production through suppression of gluconeogenesis and enhancing insulin suppression of endogenous glucose production and, to a lesser extent, by reducing intestinal glucose absorption and possibly improving glucose uptake and utilization by peripheral tissues, such as skeletal muscle and adipose tissue [(50)].

The metformin-induced inhibition of hepatic gluconeogenesis has been ascribed to several mechanistic cascades. Potential mechanisms are the direct inhibition of gluconeogenic enzymes (e.g. phosphoenolpyruvate carboxykinase, fructose-1,6-bisphosphatase, and glucose- 6-phosphatase), the reduced hepatic uptake of substrates for gluconeogenesis [(51,52)]. Other investigators have also demonstrated the inhibition of mitochondrial respiration by metformin, which may reduce the energy supply required for gluconeogenesis [(53)].

1.6.2 Sulfonylureas

This class of drugs had been introduced in the 1950's and has played an important role in the management of type 2 diabetes since its introduction to the market [(54)]. The sulfonylurea drug class has evolved as different generations of agents. The first generation sulfonylureas are the oldest, and include tolbutamide, tolazamide, chlorpropamide and acetohexamide. The second-generation sulfonylureas include glyburide (also known as glibenclamide), glipizide, gliclazide and glimepiride. All sulfonylureas have the same mechanism of action; however, the second-generation agents are more potent than the first-generation agents on a weight basis [(55)]. In terms of clinical response, the sulfonylureas, on average, lower HbA1c by 1–2% [(56)]. As insulin secretagogues, the pharmacodynamic effects of sulfonylureas are dependent on functioning β cells; thus they are typically used earlier in the course of the disease

process. In later stages of the disease, when β-cell dysfunction becomes most prevalent, sulfonylurea efficacy may decline and may necessitate an increase in drug dosage or the switch to other non-insulin secretagogue therapy [57].

1.6.2.1 Glibenclamide

Glibenclamide also known as glyburide, is an oral hypoglycemic of the sulfonylurea group that is frequently prescribed, for the treatment of noninsulin dependent diabetes mellitus [58]. Chemically is 5-chloro-N-[4 [[[(cyclohexylamino)carbonyl]amino]sulphonyl]phenyl] ethyl]-2 methoxy benzamide [59] Figure(1.3).

The main effect of sulfonylureas is the rise in plasma insulin concentrations; consequently they are effective only when residual pancreatic β-cells are present. The rise in plasma insulin levels occurs for two reasons. Firstly, there is stimulation of insulin secretion by pancreatic β-cells, and secondly, there is a decrease in hepatic clearance of insulin [60].

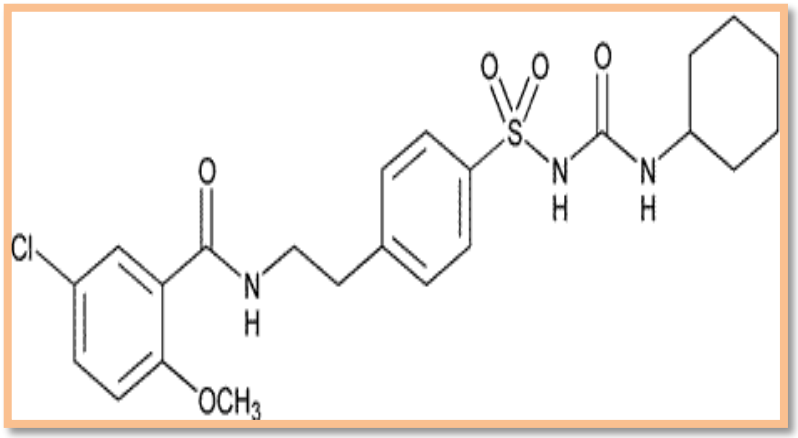


Figure 1.3 Chemical structure of Glibenclamide [58].

1.6.2.2 Mechanisms of actions of sulfonylureas

Sulfonylureas improve glucose levels by stimulating insulin secretion by binding to the specific receptor for sulfonylureas on β-pancreatic cells, blocking the inflow of potassium (K+) through the ATP-dependent channel: the flow of K+ within the β-cell goes to zero; the cell membrane becomes depolarized, thus removing the electric screen which prevents the diffusion of calcium into the cytosol. The increased flow of calcium into β-cells causes the contraction of the filaments of actomyosin responsible for the exocytosis of insulin, which is therefore promptly secreted in large amounts [60,61] Figure (1.4).

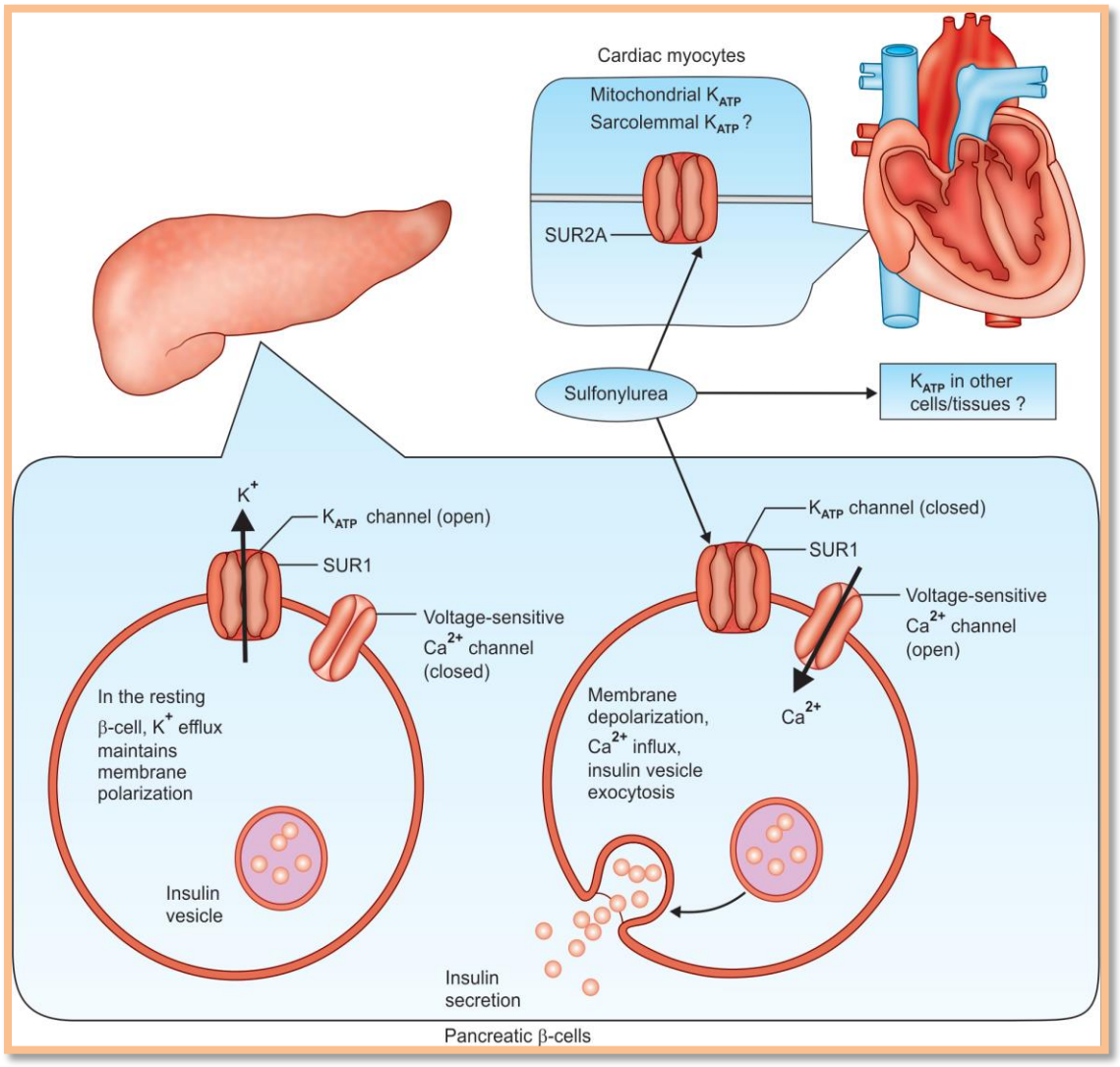


Figure (1.4): Mechanism of action of sulfonylureas [62] .

1.6.3 Combination Therapy

To maintain normal blood glucose levels in patients having type-II diabetes mellitus who have not been stabilized on monotherapies, a combination of Metformin & Glibenclamide tablets were prepared with the aim to minimize the risk of long-term complications by decreasing the polypharmacy [63,64]. Combination of glibenclamide and metformin hydrochloride simultaneously addresses two different but complimentary mechanisms to improve glycemic control in type 2 diabetes [65].

1.7 Leptin Hormone

1.7.1 Structure , Synthesis and Biological Functions of Leptin

leptin (derived from the Greek word "leptos" meaning lean) is a peptide hormone contains 167 amino acids, which is discovered at the end of the year 1994 [66]. It is produced by differentiated adipocytes, although production has been demonstrated in other tissues, such as the funds of the stomach, skeletal muscle, liver and the placenta [67]. Leptin acts on the central nervous system, in particular the hypothalamus, suppressing food intake and stimulating energy expenditure [68] Figure (1.5).

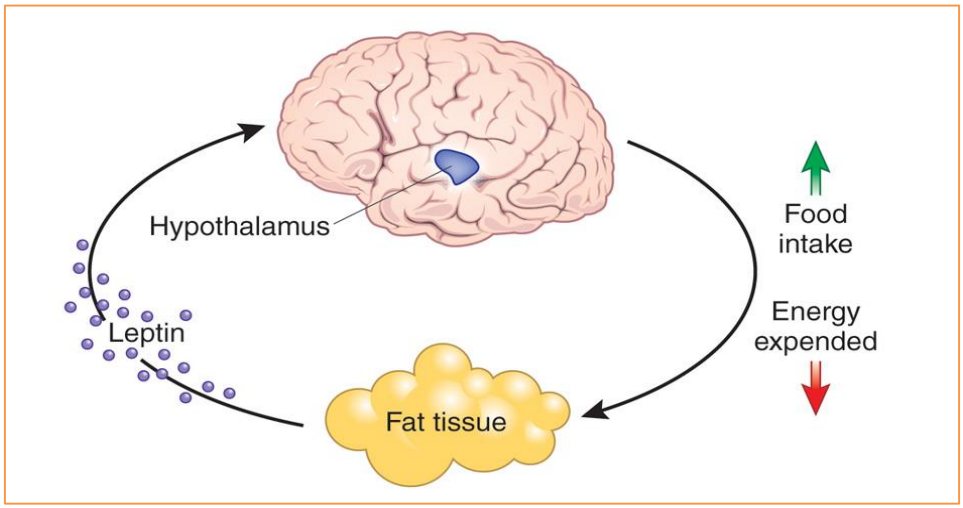


Figure (1.5): Leptin and the regulation of energy balance [67].

Leptin serves as a major 'adipostat' by repressing food intake and promoting energy expenditure. Independent of these effects, leptin improves peripheral (hepatic and skeletal muscle) insulin sensitivity and modulates pancreatic β-cell function. In the majority of cases of obesity, despite both an intact leptin receptor and high circulating leptin levels, leptin fails to induce weight loss. This diminished response to the anorexigenic and insulin sensitizing effects of leptin is called "leptin resistance" [(69)].The rising prevalence of human obesity and type 2 diabetes has generated intense interest in the physiological roles that leptin plays in energy balance and food intake regulation [(70,71)] .

1.7.2 Leptin hormone and Type 2 diabetics

The development of Type 2 diabetes in association with obesity, hyperinsulinemia and insulin resistance has been demonstrated. Obesity is associated with a marked increase in circulating leptin concentration [(72)]. However, plasma leptin displays a strong correlation with insulin concentration, insulin resistance, metabolic syndrome, dyslipidemia, even after controlling for measures of body fat mass [(73)]. Leptin, on the other hand, has been shown to improve insulin sensitivity and glucose metabolism in leptin treated rats [(74)] and a similar response has been reported in human[(75)].However, relationship between leptin concentrations and insulin resistance has also been cited [(76)]. Therefore it is evident that leptin's influence on insulin action is as debatable as insulin's effect on leptin levels. The different severity or condition of type 2 diabetes might result in different degree of insulin concentration and resistance. Many studies have demonstrated that taking oral antidiabetic drugs might change the plasma leptin concentration in type 2 diabetes [(77,78)].

Leptin receptors that are found in pancreatic β-cells raise the possibility that leptin may modulate insulin secretion. Exogenous leptin lowers plasma insulin levels and in vitro, leptin suppresses insulin release in human islet cells. There is evidence to suggest that leptin may play a role in the pathophysiology of diabetes, possibly by suppressing insulin secretion. Since elevated baseline insulin is associated with both diabetes risk and elevated leptin levels, it could confound an association between leptin levels and diabetes [(79)].

1.8 Glucose Regulation

Changes in serum glucose levels are a result of the following process: release of stored glucose through hepatic glycogenolysis, and creation of glucose from non-glucose sources through hepatic gluconeogenesis. Glycogenolysis releases glucose through breakdown of glycogen, the stored form of glucose, and gluconeogenesis creates glucose from amino acid and lactate. During periods of fasting, glucose levels are regulated by glycogenolysis and gluconeogenesis[(80)].

The liver plays an important role in the regulation of blood glucose levels. At time of stress or whenever blood glucose fall, the liver rapidly releases glucose in to the blood stream, which carries it to other tissues (e.g., brain and muscle) that cannot make glucose [(81)] Figure (1.6).

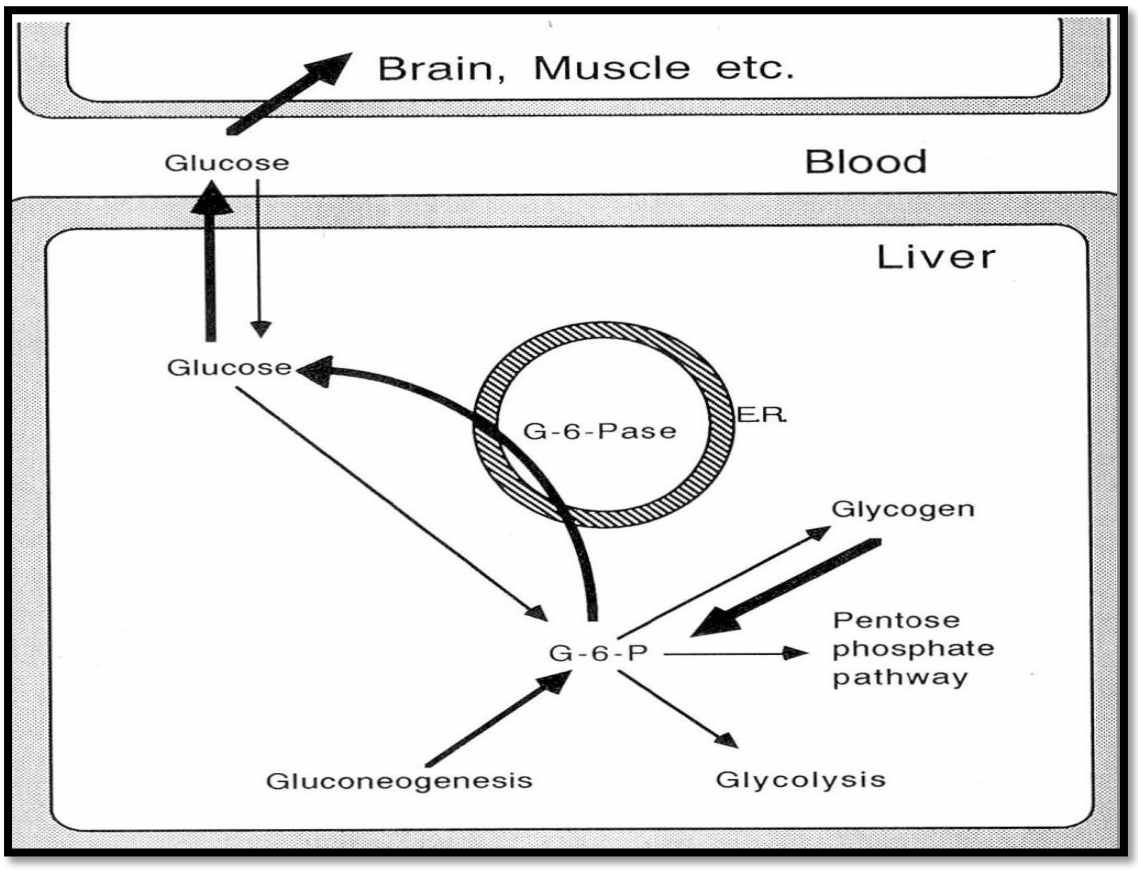


Figure (1.6): schematic representation of the pathways of hepatic glucose metabolism **(82).**

The two pathways by which the liver can make glucose are gluconeogenesis and glycogenolysis one enzyme, Glucose-6-phosphatase (G-6-Pase),catalyzes the terminal step of both pathways **(83).**The importance of G-6-Pase in the maintenance of blood glucose levels first became obvious in 1952 when G-6-Pase enzyme activity was shown

To be absent in patients with the severe metabolic disorder that is now termed classical type 1a glycogen storage disease **(84).**

1.8.1 Glucose-6-phosphatase Enzyme

Glucose-6-phosphatase (G-6-Pase), a key enzyme in glucose homeostasis catalyzes the terminal enzymatic step of both gluconeogenesis and glycogenolysis by converting glucose-6-phosphate (G-6-P) to glucose and inorganic phosphate. Inhibition of the G-6-Pase system in the liver is expected to result in a reduction of hepatic glucose production irrespective to hepatic glucose output [(85)]**.** In healthy subjects, hepatic gluconeogenesis and glycogenolysis contribute approximately equally to basal endogenous glucose production [(86,87)]**.**

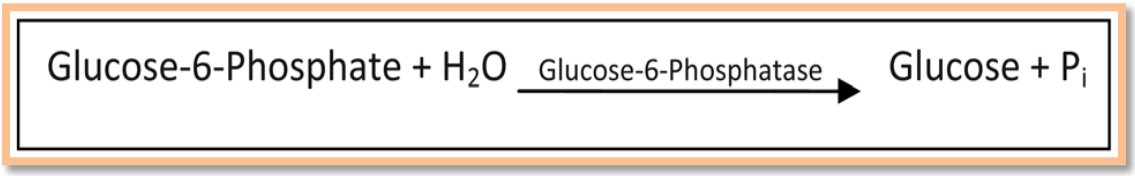
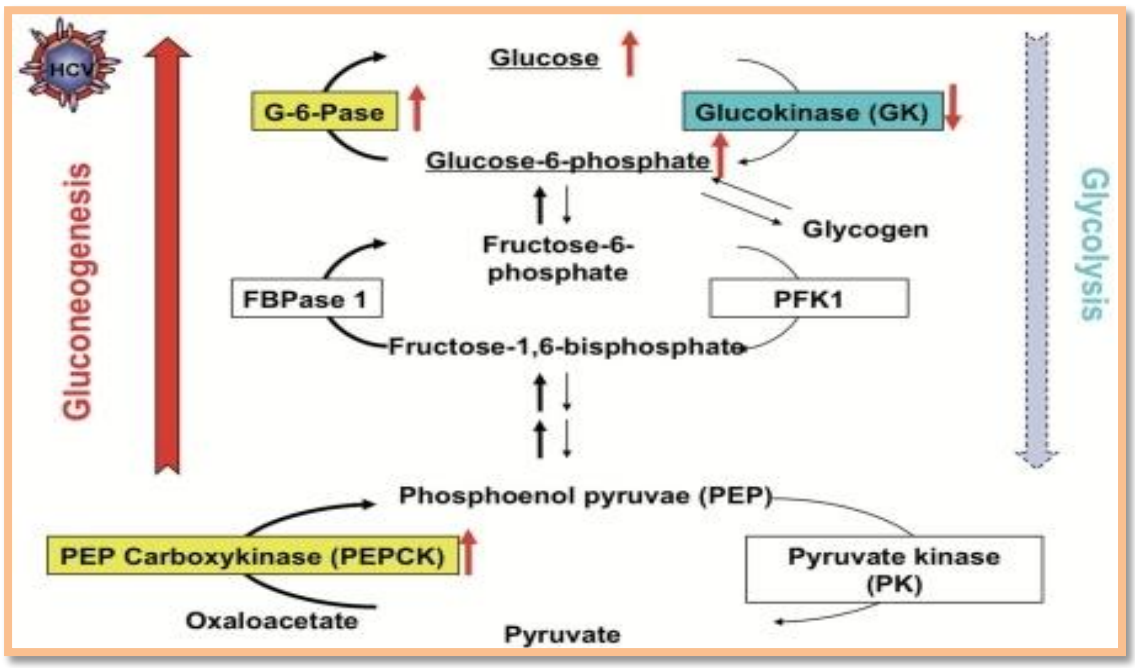


Figure (1.7): Glucose-6-phosphatase reaction [(83)].

Glucose-6-phosphatase is most abundant in liver tissue, but also present in kidney cells, small intestine, pancreatic islets and at a lower concentration in the gallbladder plays the important role of providing glucose during starvation [(88)]**.** G-6-Pases is highly hydrophobic proteins consist of 357 amino acid residues tightly associated with the endoplasmic reticulum (ER) membranes. Human G-6-Pase is anchored to the ER by 9 transmembrane helices with the amino (N) terminus in the lumen and the carboxyl (C) terminus in the cytoplasm [(89)]**.**

1.8.2 Role of Glucose-6-Phosphatase in hepatic glucose production and Type 2 Diabetes mellitus

Type 2 diabetes mellitus is characterized by fasting hyperglycemia and an excessive, prolonged rise in the plasma glucose concentration after glucose or meal ingestion. The fasting hyperglycemia has been attributed to result mostly from an increased rate of glucose production, which could result from an increased rate of hepatic glycogenolysis and gluconeogenesis [(90)]. The overall hepatic glucose output was an increase by twofold and the gluconeogenesis more than threefold in patients compare with the controls. This finding demonstrated the increased in gluconeogenesis is the predominant mechanism responsible for increased hepatic glucose output in patients with T2DM and it is correlated with fasting plasma glucose level [(91)] .Glycogenolysis occurs within 2-6 h after a meal in humans, and gluconeogenesis has a greater importance with prolonged fasting. The rate of gluconeogenesis is controlled principally by the activities of unidirectional enzymes such as phosphoenolpyruvate carboxykinase (PEPCK), fructose-1, 6-bisphosphatase and glucose-6-phosphatase (G-6-Pase). PEPCK catalyzes one of the rate limiting steps of gluconeogenesis, the conversion of oxaloacetate to phosphoenolpyruvate (PEP), while G-6-Pase catalyzes the final step of gluconeogenesis, the production of free glucose from glucose-6-phosphate (G6P) [(92)] Figure(1.8).



Figure(1.8): Gluconeogenesis pathway[92].

The genes of these gluconeogenic enzymes are controlled at the transcriptional level by hormones, mainly insulin, glucagon and glucocorticoids [93]**.** In both type 1 and type 2 diabetes, excessive hepatic glucose production is a major contributor of both fasting and postprandial hyperglycemia [94]**.** Enzymes that have high control strength on hepatic glucose metabolism are potential targets for controlling hepatic glucose balance and thereby glucose levels in type 2 diabetes [95]**.** The inhibition of enzymes involved in glycogenolysis constitutes an alternative approach to suppressing hepatic glucose production and lowering blood glucose levels [96,97]**.** Hepatic glycogen phosphorylase (GP) and glucose-6-phosphatase (G-6-Pase) are two key enzymes in glycogenolysis. GP catalyzes the first step of the breakdown of glycogen to yield glucose-1- phosphate, whereas G-6-Pase catalyzes the final reaction in hepatic glucose production Figure (1.9) [98]**.**

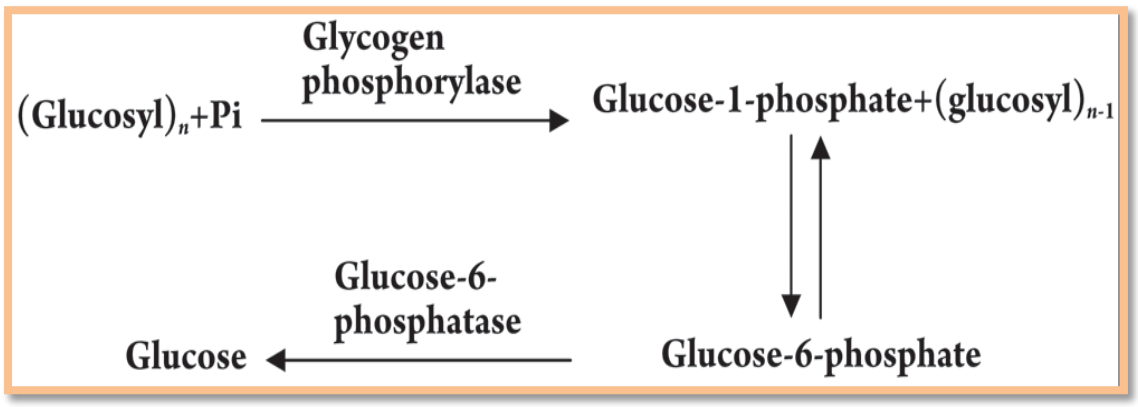


Figure: (1.9) Pathways of glycogen metabolism in the liver.

Both enzymes have been proposed as potential targets for anti hyperglycemic drugs for diabetes and the correlation between type 2 diabetes and glucose-6-phosphatase makes this enzyme an appealing drug target for control of blood glucose levels as its inhibition would directly prevent the release of free glucose into the bloodstream [(99)].

1.9 Lipid Profile

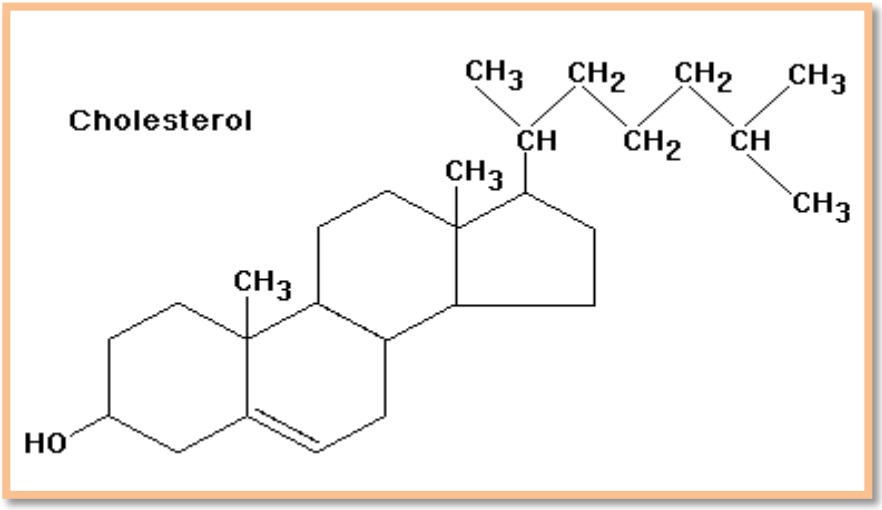
Diabetes mellitus exerts some of the most profound effect on lipid metabolism so the concentration of serum lipid (total cholesterol, HDL, LDL, VLDL, and triglyceride) is another important index of the overall metabolic control in diabetic patients [(100)]. Lipid abnormalities significantly contribute to the increased risk of cardiovascular disease and other morbidity in diabetes. There is a growing body of evidence showing that hyperglycemia and dyslipidemia are linked to increased cardiovascular risk [(101)].The prevalence of dyslipidemia in diabetes mellitus is 95% [(102)].

1.9.1 Lipoproteins

The major plasma lipids are not circulating free in the blood. They are bound to a specific protein, (apportions) to form large spherical complex molecule called lipoprotein, which are transported through plasma. There are three kinds of lipoproteins : high density lipoprotein (HDL) , low density lipoprotein (LDL) ,and very low density lipoprotein (VLDL) [(103)].

1.9.2 Total Cholesterol (TC)

Cholesterol, a waxy substance produced by the liver and found in certain food, is needed to make vitamin D and some hormones, builds cell walls, and creates bile salts that help to digest fat. Cholesterol has a chief role in pathologic processes as a factor in the genesis of atherosclerosis of vital arteries, causing cerebrovascular, coronary, and peripheral vascular disease. However, cholesterol is found as a chief of several lipoproteins (HDL) [(104)] Figure(1.10). The serum total cholesterol is the sum of several types of cholesterol including LDL-cholesterol, HDL-cholesterol and VLDL-cholesterol [(105)].



Figure(1.10): Structure of Cholesterol [(105)].

1.9.3 High -Density Lipoprotein (HDL –Cholesterol)

High density lipoprotein is a class of lipoproteins produced by the liver and intestines Figure(1.11). Sometimes referred to as "good cholesterol" lipoprotein [106] . HDL promotes expulsion of cholesterol from peripheral cells and, indirectly from the body thus; protecting against cardiovascular disease. several studies of large populations have shown that the risk of developing manifestation of ischemic heart disease is inversely related to the serum concentration (HDL-cholesterol) [107,108] In diabetic patients the incidence of ischemic heart disease and other atherosclerosis disease is greater than that of general population [109].

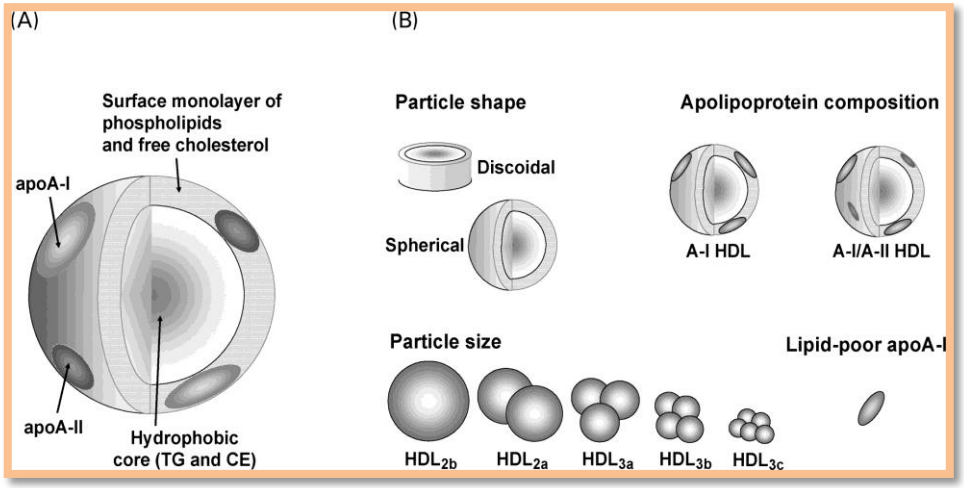


Figure (1.11): HDL Structure [109]

1.9.4 Low –Density Lipoprotein (LDL-Cholesterol)

The role of the LDL-C in the blood is to transport cholesterol to the peripheral tissue [110] , making it available to the tissue cells for membrane or hormonal synthesis and for storage for later use. This type of cholesterol is considered harmful as it transports a large amount of cholesterol [111]. The development of cardiovascular disease in Type 2 DM is often predicted by several factors which include elevated low-density lipoprotein (LDL-C) levels [112].

1.9.5 Very Low Density Lipoprotein Cholesterol (VLDL-C)

Very low Density Lipoprotein (VLDL) are synthesized in the liver and transport endogenous triglyceride from the liver to other tissues [(113)]. VLDL-C are hydrolyzed by the action of lipid protein lipase by this process VLDL are converted to IDL which is removed by liver but most have more triglyceride removed by hepatic lipase and is there by converted into LDL Excess dietary intake of carbohydrate, saturated fatty acids, and trans fatty acids enhances the hepatic synthesis of triglycerides that , in turn , increases VLDL production [(114)].

1.9.6 Triglyceride

High triglyceride level is often associated with insulin resistance and hypertension, producing a constellation of condition of very high risk for macro vascular disease (metabolic syndrome). The latter reaches its full expression in patients with type 2 diabetes. Development of insulin resistance in type 2 diabetes may be due to alteration in the partitioning of fat between the adiposity, muscle, and liver leading to accumulation of triglyceride in the latter tissues with subsequent impairments of insulin action. NIDDM is commonly associated with hypertriglyceridemia Abnormality of immune system may be a contributor to the hypertriglyceridemia [(115)] Figure (1.12).

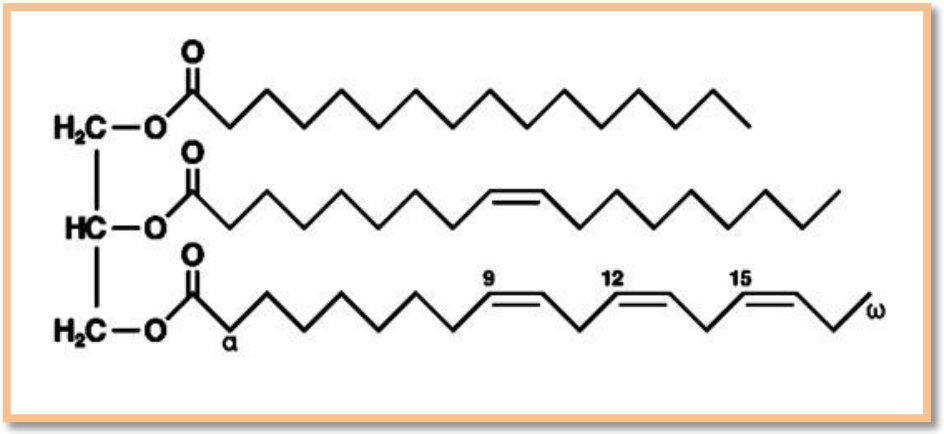


Figure (1.12): Structure of Triglyceride [(116)].

Aims of the study

The current study is conducted to achieve the following:

Part one of this study

1. To find out the levels of Glucose-6-phosphatase activity and leptin hormone in the serum of type 2 diabetic subjects and to find out whether those levels are changed in diabetic subjects that taking antidiabetic drugs such as (metformin and glibenclamide).

2. To find the correlation between Glucose -6- phosphatase activity and the serum leptin hormone, FPG and HbA1C% in four groups under the study(control group ,newly diagnosed group ,metformin group and metformin plus glibenclamide group) .

3. To find the correlation between leptin hormone and FPG ,HbA1C% ,BMI and the serum lipid profile in four groups under the study(control group ,newly diagnosis group ,metformin group and metformin plus glibenclamide group) .

In part two of the study:

Binding study of Glucose-6-phophatase with antibody (biotin antibody) in sera of newly diagnosed group ,metformin group and metformin plus glibenclamide group and study the effect of various factors on this binding like [Antibody concentration, enzyme concentration, temperature and incubation time] .

Chapter two

Samples and Methods

2.1 Patients and Control

Sixty-four patients with type 2 diabetes mellitus were selected according to convenient non-random one and carried out by consecutive pooling of diabetic patients attending the National center of Diabetes in (AL-Mustansiria University) during the period from November 2014 to March 2015 , with type of drug divided patients to three groups (group one: newly diagnosed (without therapy)(23), group two: with metformin therapy(MT.)(20); group three with metformin plus glibenclamide (MT. plus Glib.)(21) and 20 Healthy subjects were included in the study as a control group. Patients with renal failure, Cushing syndrome or hepatic diseases were excluded from the study after the clinical evaluation. Patients taking oral hypoglycemic agents other than metformin or glibenclamide and those taking drugs that may affect the results of the study had also been excluded. The sample for the assay were taken early in the morning between (8.30 and 11.00 A.M) while both patient and healthy subjects were relaxed and fasting for (12-14) hours.A careful history was obtained from patients including age, duration of diabetes, duration of taking treatment, family history, weight and height, type of treatment ,other diseases and smoking.

All patients were clinically examined, Pregnant patients were not enrolled. Evaluation of each patient is done by detecting the body mass index (BMI), levels of fasting plasma glucose(FPG), glycated hemoglobin HbA1c%, lipid profile , glucose-6-phosphatase activity(G-6-Pase),and concentration of leptin hormone.

2.2 Collection of Blood samples

From each subjects, 10 mL of blood were obtained by vein puncture, using a 10 ml disposable syringes. The blood sample was divided into two aliquots; 2 & 8 ml. the first aliquot blood was dispensed in a tube containing Ethylene Diamine Tetraacetic Acid (EDTA),this blood mixed gently and used for HbA1c estimation, While the second aliquot was dispensed in a plain tube and left to clot at room temperature (25 °C), and then separated by centrifuge at (3000 rpm) for (10 min) to collect serum.

The serum was divided into two Eppendorf tubes and stored in the deep Freeze (-20 ^{0}C) until the assay day.

Materials:

2.3 Kits and instruments:

Kits were used from different sources are listed in Table (2.1), instruments that had been used for various measurements are listed in Table(2.2).

Table (2-1): Kits companies, and country of manufacture.

Chemicals	Suppliers
Glucose	Spinreact, Spain
Glucose-6-phophatase Enzyme ELISA	Cusabio, China
Glycated hemoglobin (GHb)	Infopia, Korea
HDL-Cholesterol	Randox, U.K
Leptin (Sandwich) ELISA	DRG Instruments GmbH, Germany
Total Cholesterol	Randox, U.K
Triglyceride	Randox, U.K

Table (2.2) Instruments used and the companies supplied them.

Instruments	Suppliers
Centrifuge	Hittich Universal-Germany
Incubator	Germany
Length scale	Salter ,England
Micro ELISA system (washer&reader)	(Thermo,Germany)
Micropipettes and multichannel	Gilson, France.
Shakers	Kahn Technolabo ,Italy
Spectrophotometer	Cecil CE 72000,France
Timer with alarm	Junghans,Germany
Weight scale	Raven equipment limited,England

2.4 Methods and Biochemical Determination of BMI, FPG, HbA1c % for the three groups T2DM and controls:-

2.4.1 Measurement of Body Mass Index (BMI) for the three groups T2DM and controls

BMI uses a mathematical formula based on a person's height and weight. BMI equals weight in Kilograms divided by height in square meter ($BMI=Kg/m^2$) [117]. [WHO; and Fost] suggested that a BMI of 18.5–24.9 indicates a person of normal weight. A person with a BMI of 25–29.9 is overweight while a person with a BMI of ≥ 30 is obese [118].

2.4.2 Measurement of Fasting Plasma Glucose (FPG) for the three groups T2DM and controls

2.4.2.1 Principle:-

Glucose level has been evaluated according to the method of Barham and Trindoer (1972) [119], which is based on enzymatic oxidation of glucose by glucose oxidase to gluconate with libration of hydrogen peroxide and then the reaction of peroxide with phenol to form quinonimine which is detected spectrophotometrically at 505 nm according to the following equation :-

$$\text{Glucose} + H_2O + O_2 \xrightarrow{\text{Glucose Oxidase}} \text{Gluconate} + O_2 + H_2O_2$$

$$H_2O_2 + \text{Phenol} + 4 - \text{amionantipyrin} \xrightarrow{\text{peroxidase}} H_2O + \text{Quinoneimine}$$

2.4.2.2 Reagent composition:

Reagent1 buffer	TRIS PH7.4 9.2 mol/L Phenol 0.3 mmol/L
Reagent2 Enzymes	4-aminopenazone 2.6 mmole/L Glucose oxidase 15000 U/L Peroxidase 1000 U/L
Glucose CAL	Glucose aqueous primary standard 100 mg/dL

2.4.1.3 Reagent preparation:

Working reagent was prepared by dissolving the continents of one vial R2 Enzymes in one bottle of R1Buffer. Then was capped and was mixed gently to dissolve contents.

2.4.2.3 Procedure:

1. One ml of working reagent was placed into blank, standard and sample tubes.

2. Ten micro liters of standard were placed into the standard tube .

3. Ten micro liters of group1 serum were placed on the sample tube.

4. Tubes were mixed and incubated for 10 min at room temperature.

5. Absorbance was read of the samples and standards against the blank at 505nm.

- Same steps mention above were repeated to determine the concentration of FPG in group 2,group3 and control group.

2.4.2.4 Calculation:

$\frac{(A)\text{sample}}{(A)\text{standard}} \times 100(\text{ standard conc.}) = \text{mg/dL}$.

Conversion factor: mg/dL x 0.0555= mmol/L.

N.V (FPG) = 65 - 110 mg/dl = 3.6 - 6.1 mmol/L **.**

2.4.3 Measurement of Glycated Hemoglobin (GHb or HbA1C) for the three groups T2DM and controls

2.4.3.1 Test components

1.Cartridge.

2.Reagent pack: has 2 chambers to be inserted into the cartridge.

Chamber 1 (reagent solution)contains: 25 v/v % boronate affinity bead, surfactant, nonreactive ingredients, buffer.

Champer 2 (rinsing solution) contains: surfactant, nonreactive ingredients, buffer.

2.4.3.2 Procedure:

Glycosylated or glycated hemoglobin is a form of hemoglobin used primarily to identify the average plasma glucose concentration over prolonged periods of time [(120)] .The CLOVER A1c system is a fully automated boronate affinity assay for the determination of the percentage of Hemoglobin A1c (HbA1c%) in whole blood .The CLOVER A1C test cartridge includes a cartridge and a reagent pack. The reagent pack is pre-filled with reagent solution and rinsing solution. The reagent solution contains agents that lyse erythrocytes and boronate bead that binds cis diols of glycated hemoglobin. The blood sample size of 4 µL is obtained with the capillary tip of the reagent pack. By inserting the reagent pack into the cartridge, the blood is instantly lysed releasing the hemoglobin and the boronate bead binding the glycated hemoglobin.

The blood sample mixture is rotated to the measurement zone of the cartridge, whereas the amount of total hemoglobin in the blood sample is measured by the reflectance of the photo sensor, which consists of LED (Light Emitting Diode) and PD(Photo diode),the cartridge is then rotated

so that the rinsing solution wash out non-glycated hemoglobin from the blood sample, thus the amount of glycated hemoglobin can be photometrically measured. The ratio of glycated hemoglobin with respect to the total hemoglobin in the blood sample is calculated.

$$\text{HbA1C\%} = A \times \left[\frac{\text{HbA1c}}{\text{Total hemoglobin}} \times 100 \right] + B$$

Where 'HbA1c' and 'Total Hemoglobin' are the signals obtained from the CLOVER A1c™ Self system, 'A' and 'B' are the slope and intercept factor to correct the value for DCCT(Diabetes Control and Complication Trial) calibration [121].

2.5 Serum Lipid Profile Assay

2.5.1 Measurement of Serum Total Cholesterol (TC) for the three groups T2DM and controls:-

2.5.1.1 Principle:-

Total serum cholesterol was determined to utilizing a readymade laboratory kit for this purpose, the principle of determination was based on the enzymatic hydrolysis. In the presence of cholesterol esterase, the cholesterol esters in the sample are hydrolyzed to cholesterol and free fatty acid. The cholesterol produced is oxidized by cholesterol oxidase to Cholesterol-3-one and hydrogen peroxide is then detected by a chromogenic oxygen acceptor, phenol aminopyrine, in the presence of peroxidase [122,123].

$$\text{Cholesterol esters} + H_2O \xrightarrow[\text{Esterase}]{\text{Cholesterol}} \text{Cholesterol} + \text{Fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow[\text{Oxidase}]{\text{Cholesterol}} \text{Cholestrol–3 – one} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + \text{4-Amino phenazone} \xrightarrow{\text{Peroxidase}} 4H_2O + \text{Quinonimine}$$

The quantity of the formed red dye quinonimide is proportional to the cholesterol concentration. The absorbance of quinonimine was read at 505 nm spectrophotometer**.**

2.5.1.2 Reagent composition:

Reagent 1 buffer	PIPES pH 6.9 90 mmol/L Phenol 26 mmol/L
Reagent 2 Enzymes	Cholesterol esterase(CHE) 300 U/L Chol. oxidase(CHOD) 300U/L Peroxidase (POD) 1250U/L 4-Aminophenazone(4-Ap) 0.4 mmol/L
Cholesterol CAL	Cholesterol aqueous primary standard 200 mg/dl

2.5.1.3 Reagent preparation:

Working reagent was prepared by dissolving the continents of one vial R2Enzymes in one bottle of R1Buffer. Then was capped and was mixed gently to dissolve contents.

2.5.1.4 Procedure:

1. One ml of working reagent was placed into blank, standard and sample tubes.

2. Ten micro liters of standard were placed in to the standard tube.

3. Ten micro liters of group1 serum were placed on the sample tube.

4. Tubes were mixed and incubated for 10 min at room temperature.

5. Absorbance was read of the samples and standards against the blank at 505nm.

- Same steps mention above were repeated to determine the concentration of cholesterol in group 2,group3 and control group.

2.5.1.5 Calculation:

$$\frac{(A)\text{sample}}{(A)\text{standard}} \times 200(\text{standard conc.}) = \text{mg/dL cholesterol in the sample}$$

Conversation factor: mg/dL x 0.0258 = mmol/L

2.5.2 Measurement of Serum Triglyceride (TG) for the three groups T2DM and controls:-

2.5.2.1 Principle:-

The triglycerides were determined by enzymatically hydrolyzed glycerol and fatty acids according to the following equations:- [124]

$$TG + 3H_2O \xrightarrow{\text{Lipase}} \text{glycerol} + \text{fatty acids}$$

Glycerol + ATP $\xrightarrow{\text{Glycerol kinase}}$ glycerol-3-phosphate +ADP

Glycerol-3-phosphate $\xrightarrow{\text{Glycerol-3-phosphate oxidase}}$ dihydroxy acetone phosphate + H_2O_2

$2H_2O_2$ + parachlorophenol +4-amino antipyrine $\xrightarrow{\text{Peroxidase}}$ Quinoneimine $+4H_2O$

2.5.2.2 Reagents Composition:

Reagent 1 Buffer	pH 7.5 50mmol/L p-chlorophenol 2mmol/L
Reagent 2 Enzymes	Lipoprtien lipase (LPL)150000 U/L Glycerolkinase(GK) 500 U/L Glycerol-3-oxidase(GPO)2500 U/L Peroxidase (POD) 440U/L 4-Aminophenazone(4-AP) 0.1mmol/L ATP 0.1mmol/L
Triglycerides CAL	Triglycerides aqueous primary standard 200mg/dl

2.5.2.3 Reagents Preparation:

Working reagent was prepared by dissolving the contents of one vial R2 Enzymes into one bottle of R1 Buffer, then was capped and was mixed gently to dissolve contents .

2.5.2.4 Procedure.

1. One ml of working reagent was placed into blank, standard, and sample tubes.

2. Ten micro liters of standard were placed on the standard tube.

3. Ten micro liters of group1 serum were placed on the sample tube.

4. Tubes were mixed and incubated for 10 min at room temperature.

5. Absorbance was read of the samples and standard against the blank at 505nm.

- Same steps mention above were repeated to determine the concentration of triglyceride in group 2,group3 and control group.

2.5.2.5 Calculation:

$$\frac{\text{(A) sample}}{\text{(A) standard}} \times 200 \text{ (standard conc.)} = \text{mg/dl triglycerides in the sample}$$

Conservation factor: mg/dL x 0.0113= mmol /L

2.5.3 Measurement of Serum High Density Lipoprotein-Cholesterol (HDL–C) for the three groups T2DM and controls:-

2.5.3.1 Principle:-

Low density lipoprotein (LDL), Very Low Density Lipoprotein (VLDL), and chylomicron fraction are precipitated quantitatively by the addition of phosphotungstic acid which contains magnesium chloride at pH 6.2. After centrifugation, the supernatant contains cholesterol concentration in the HDL fraction which is determined by using cholesterol kit [125,126].

2.5.3.2 Reagents Composition:

Reagent	phosphotungstic Acid 0.55 mmol/L Magnesium chloride 25 mmol/L
CAL standard	HDL- cholesterol precipitant standard

2.5.3.3 Procedure.

1. Five hundred micro liters of group1 serum and standard were placed in to sample and standard tubes.

2. One ml of reagent was placed into the sample, standard tubes.

3. Tubes were mixed and incubated for 10 min at room temperature, then were centrifuged for 10 min at 4000 rpm .

4. The supernatant within two hours was read and determine the cholesterol content.

5. Absorbance was read at λ500nm.

- Same steps mention above were repeated to determine the concentration of HDL-C in group 2,group3 and control group.

2.5.3.4 Calculation

$$\frac{(A)\ \text{sample}}{(A)\ \text{standard}} \times \text{conc. of standard} = \text{mg/dl HDL-C in the sample}$$

Conversation factor: mg/dL x 0.0258 = mmol/L

2.5.4 Measurement of Serum Low Density Lipoprotein Cholesterol (LDL–C) for the three groups T2DM and controls:-

LDL-cholesterol is very difficult to isolate and measure.Hence, LDL level is most usually derived by the **Friedwalds formula** as follows [127]

LDL- cholesterol = Total cholesterol – [HDL- cholesterol + TG/2.2]

2.5.5 Measurement of Serum Very Low Density Lipoprotein (VLDL) for the three groups T2DM and controls:-

Very low-density lipoprotein- cholesterol was estimated by using formula of Friedwald [127].

[VLDL-Cholesterol] = TG/2.2

2.6 Determination of glucose-6-phosphatase activity in sera of three groups T2DM and controls:

2.6.1 Quantitative determination of human glucose-6-phosphatase (G-6-Pase) by ELISA Test:

Principle :

This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for G-6-Pase has been pre-coated onto a microplate. Standards and samples are pipettes into the wells and any G-6-Pase present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for G-6-Pase is added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of G-6-Pase bound in the initial step. The color development is stopped and the intensity of the color is measured.

2.6.2 Reagents

1.Standards (standard 0-7) : concentration :0, 0.78, 1.56, 3.12, 6.25, 12.5, 25,50 U/ml.

2.Biotin-antibody and Biotin-antibody Diluent.

3.HRP-avidin and HRP-avidin Diluent.

5.Substrate solution: Tetramethylbenzidine (TMB)

6.Sample Diluent.

7.Washing Buffer.

8.Stopping Solution.

9.Adhesive strip(for 96 wells).

2.6.3 Sample preparation

Serum samples require a 4-fold dilution into Sample Diluents.

2.6.4 Procedure

1. One hundred µls of standard and serum were added per well. Covered with the adhesive strip provided and Incubated for 2 hours at 37°C.

2. The liquid of each well was removed , without washing.

3. One hundred µls of Biotin-antibody(1x)were added to each well. Covered with a new adhesive strip and Incubated for 1 hour at 37°C.

4. Microtiter wells were rinsed 3 times with washing solution, and strike sharply onto absorbent paper to remove all residual water droplets.

5. One hundred µls of HRP-avidin (1x)were added to each well. Covered the microtiter plate with a new adhesive strip and Incubated for 1 hour at 37°C.

6. The aspiration/wash process was repeated for five times as in step 4.

8. Ninty µls of TMB Substrate were added to each well and incubated for 15-30 minutes at 37°C. (Protect from light).

9. Fifty µls of stop solution were added to each well and gently tap the plate to ensure thorough mixing

10. The absorbance(optical density) of each well was determined within 5 minutes, using a microplate reader set to 450 nm.

2.6.5 Calculation: The absorbance value for each set (references standards and samples) was calculated for 450nm. A standard curve was constructed by plotting the absorbance which were obtained from reference standard against their activity in U/ml on linear graph paper. Absorbance values on the vertical or Y-axis and activities on the horizontal or X-axis.Then from standard curve the corresponding activity of Glucose-6-phosphatase in U/ml is determined by using the absorbance value for each sample as seen in Figure(2.1).The activity read from the standard curve must be multiplied by the dilution factor.

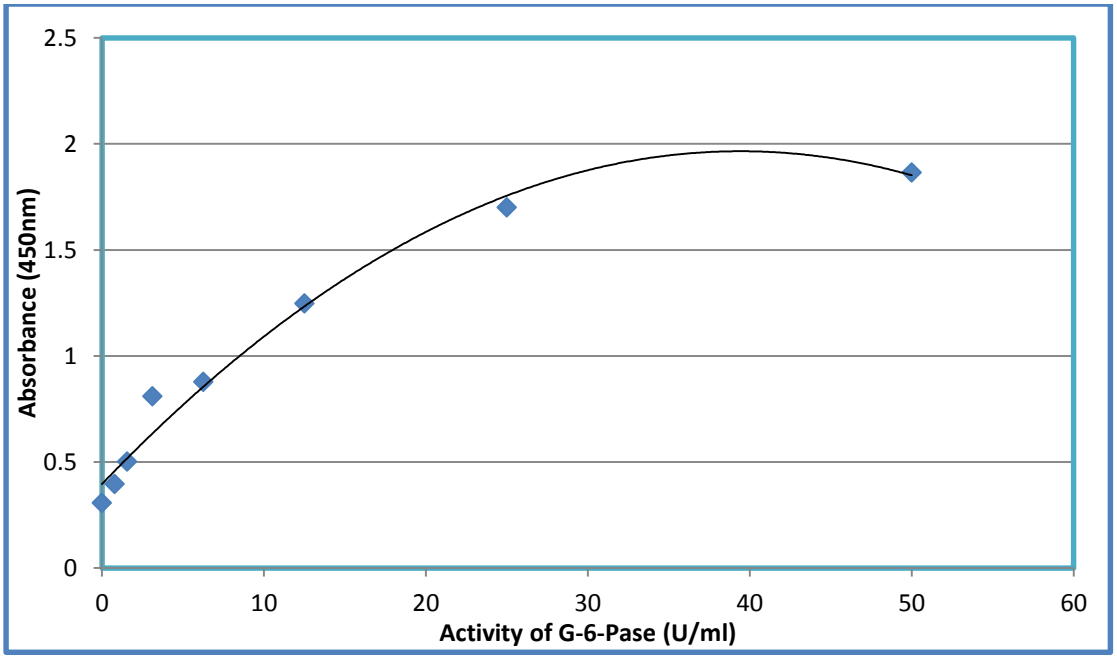


Figure (2.1): Standard curve of G-6-Pase determination in human sera by ELISA.

2.7 Determination of leptin concentration in sera of three groups T2DM and controls:

2.7.1 Quantitative determination of leptin concentrations by(ELISA) Test:

Principle :

The DRG Leptin ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle.
The microtiter wells are coated with a monoclonal antibody directed towards a unique antigenic site on a Leptin molecule. An aliquot of specimen sample containing endogenous Leptin is incubated in the coated well with a specific biotinylated monoclonal anti-Leptin antibody. A sandwich complex is formed. After incubation, the unbound material is washed off and a Steptavidin Peroxidase Enzyme Complex is added for detection of the bound Leptin. Having added the substrate solution, the intensity of colour developed is proportional to the concentration of Leptin in the specimen sample.

2.7.2 Reagents

1.Standard (Standard 0-5),6 vials, (lyophilized), 0.5 mL;
Concentrations: 0 – 2 – 5 – 25 – 50 – 100 ng/mL.
2.Control (Low & High), (lyophilized), 0.5 mL.
3.Assay Buffer: composed of 1 vial (11 mL).
4.Antiserum: composed of 1 vial (11 mL monoclonal biotinylated anti-Leptin antibody).
5.Enzyme Complex: composed 1 vial (11 mL Strepavidin conjugated to horseradish Peroxidase).
6.Substrate Solution: composed 1 vial (14 mL Tetramethylbenzidine (TMB)).

7.Stop Solution: composed 1 vial (14 mL contains 0.5 M H2SO4).

8. Wash Solution: composed 1 vial (30 mL (40X concentrated)).

Reagent Preparation

1. Standards : The lyophilized contents of the standard vials with 0.5 mL Aqua dest and let stand for 10 minutes in minimum. The vials were mixed several times.

2. Control: The lyophilized content of each vial were reconstituted with 0.5 mL Aqua dest and let stand for 10 minutes in minimum.The control vials were mixed several times before use.

3. Wash Solution: Deionized water was added to the 40X concentrated wash solution,30 mL of concentrated wash was diluted with 1170 mL deionized water to a final volume of 1200 mL.

2.7.3 Procedure

1. A volume of 15 µLs from of each standard, controls and samples with new disposable tips were added into appropriate wells.

2. One hundred µL of Assay Buffer was added to each well thoroughly mixed for 10 seconds and Incubated at room temperature for 120 minutes (without covering the plate).The contents of the wells were briskly shaken out.

3. The wells were rinsed 3 times with diluted Wash Solution (300 µL per well)and the wells were strike sharply on absorbent paper to remove residual droplets.

4. One hundred µL of antiserum were added to each well and incubated for 30 minutes at room temperature. The contents of the wells were briskly shaken out.

5. The wells were rinsed 3 times with diluted Wash Solution (300 µL per well)and the well were strike sharply on absorbent paper to remove residual droplets.

6. One hundred µL of enzyme complex were added into each well and Incubated for 30 minutes at room temperature. The contents of the wells were briskly shaken out.

7. The wells were rinsed 3 times with diluted wash solution (300 µL per well)and the well were strike sharply on absorbent paper to remove residual droplets.

8. One hundred µL of substrate solution were added to each well and Incubated for 15 minutes at room temperature.

9. Fifty µLs of stop solution were added to each well to stop the enzymatic reaction.

11. The absorbance (OD) of each well was measured at 450 ± 10 nm with a micro titer plate reader. within 10 minutes after addition of the stop solution.

2.7.5 Calculation:

The absorbance value for each set (standards, controls, and patient samples) was calculated for 450nm.A standard curve was constructed by plotting the absorbance which were obtained from each standard against their concentration in ng/ml on linear graph paper. Absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis .From standard curve the corresponding concentration of leptin hormone in ng/ml is determined by using the absorbance value for each sample as seen in Figure(2.2).

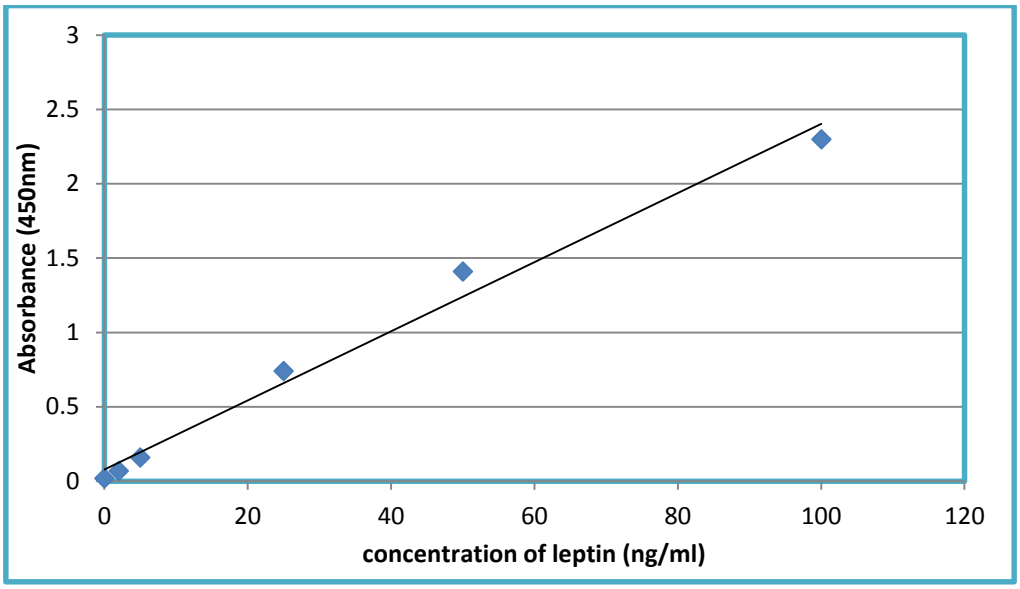


Figure (2.2) Standard curve of leptin determination in human sera by ELISA.

2.8 Binding studies of glucose- 6- phosphatase with Biotin Antibody in the sera of Patients with T2DM:

(2.8.1) The Effect of different concentrations of biotin Antibody on the binding with sera G-6-Pase:

1. A volume of (50 µls) of patients' serum (group1) was added to increasing volumes of biotin antibody (15, 25,50, 75 and 100 µls)**.**

2. The wells were mixed for 10 seconds, covered with a new adhesive strip and Incubated for 1 hour at 37°C.

3. Fifty µls of HRP-avidin were added, covered with a new adhesive strip and Incubated for 1 hour at 37°C.

4. Forty-five µls of tetramethylbenzidine (TMB) reagent were dispensed to each well**.**

5. Wells were incubated for 30 min at 37°C.

6. Twenty-five µls of stopping solution were added to each well.

7. The absorbance was read at 450 nm with a microtiter plate reader within 5 min.

- The same steps mentioned above were followed to measure the appropriate concentration of group2 ,group3 and control group.

Calculations

The absorbance values were plotted against the increasing concentration of biotin antibody.

(2.8.2) The Effect of different concentrations of sera G-6-Pase on the binding with biotin antibody:

1. A volume of (15 µls) of biotin antibody was added to increasing volumes of patients' serum (group1) (15, 25, 50, 75 and 100 µls).

2. Steps 2, 3, 4, 5,6 and 7 of the experiment (2.8.1) were repeated.

- The experiment was repeated for 75µls biotin antibody of group2 and control group.
- The experiment was repeated for 50 µls biotin antibody of group3.

Calculations

The absorbance values were plotted against the increasing concentration of G-6-Pase.

(2.8.3) The Effect of temperature on the binding of sera G-6-Pase with biotin antibody:

1. A volume of 15 µls of patients' serum (group1) was added to 15 µls of biotin antibody.

2. Steps 2, 3, 4, 5,6 and 7 of the experiment (2.8.1) were repeated**.**

3. The experiment was repeated at different temperatures (8, 23, 37,45 and 50^{o}C).

- The experiment was repeated for 50µls of group2 and control group serum added to 75µls of biotin antibody.

- The experiment was repeated for 25 µls of group3 serum added to 50µls of biotin antibody.

Calculations

The absorbance values were plotted against the different temperatures of incubation.

(2.8.4) The Effect of incubation time on the binding of sera G-6-Pase with biotin antibody:

1.A volume of 15 µls of patients' serum(group1) was added to 15 µls of biotin antibody. The wells were incubated at 45 ^{o}C for (15, 30, 60,90, and 120 min.).

2.Steps 2, 3, 4, 5,6 and 7 of the experiment (2.8.1) were repeated**.**

- The experiment was repeated with 50 µls of group2 and control group serum added to 75 µls of biotin antibody at 37 ^{o}C.

- The experiment was repeated for 25µls of group3 serum added to 50µls of biotin antibody at 37 ^{o}C.

Calculations

The absorbance values were plotted against the different times of incubation.

2.9 Statistical Analysis

Statistical analysis was performed using SPSS (Statistical Packages for Social Sciences- version 17.1) and Microsoft Office Excel (Microsoft Office Excel for windows; 2007). Data were analyzed by using One Way Analysis of Variance (ANOVA) to calculate the p-value for healthy and other patients groups. Student T-test was done also to compare means between groups. Pearson test was used to test the correlation between the assessed parameters. The results were presented as mean ± standard error. Statistical significance was considered at the level of ($p \leq 0.01$) and ($p \leq 0.05$).

Chapter three

Results and Discussion

3.1 Distribution of Groups

The present study included 84 subjects were divided into four groups Group1: newly diagnosed (without therapy) (23), group 2: with metformin therapy (MT.)(21), group 3: with metformin plus glibenclamide therapy (MT. plus Glib.)(20), and 20 Healthy subjects were included in the study as a control group. Age, Duration of diabetes, BMI, and FPG were evaluate in the Sera of Type 2 diabetic subjects and control, the result were revealed:

3.1.1 Age

The mean age for group1 was (53.65 ± 4.45) year, group2 (54.29 ± 3.47) years, group3 (53.70 ± 2.69) while the mean of the control group was (40.50 ± 8.18) years. This showed significant differences in age when comparing diabetics groups with control group. As shown in Table (3.1).

3.1.2 Body Mass Index (BMI)

Body mass index were found to be elevated in diabetic groups under the study. There were significant differences in BMI when comparing diabetics groups with control group as shown in Table (3.1). This result was in agreement with Fadhil et al (2011) who have demonstrated, that BMI was elevated markedly in diabetic patients compared with non-diabetic subjects **(128).**

3.1.3 Duration of Diabetes and duration of taking treatment

The duration of diabetes and duration of taking treatment in group2 showed a nonsignificant differences when compared with group3 as shown in table (3.1).

3.1.4 Level of fasting Plasma Glucose (FPG)

Table and figure (3.1) revealed that mean of FPG for group1 (newly diagnosed),group 2 (with metformin therapy), group 3 (with metformin plus glibenclamide therapy) and control group were (12.00 ± 2.34), (6.96 ± 1.30) ,(7.70 ± 1.23) and (4.67 ± 0.39) respectively.The difference was highly statistically significant between the four groups.In group1 FPG was higher than group2 and group3 this could be due to anti-diabetic drugs which reduce FPG. Metformin reduces blood glucose levels by inhibiting hepatic glucose production and reducing insulin resistance, particularly in liver and skeletal muscle [(129)].Glibenclamide enhancing insulin secretion from the pancreas blocking hepatic glucose production and reduce glucose levels [(130)]. There was a nonsignificant difference between group2 and group3 This result was in agreement with Marwan M. (2013) who have demonstrated that there were a nonsignificant differences between fasting glucose of metformin treated group and those treated by a combination therapy of metformin plus glibenclamide [(131)].

Table (3.1) Clinical characteristics of three groups diabetic patients and control group.

Parameters	Mean ± SD				P-value
	Control group	Group1 Newly diagnosed	Group 2 MT.	Group 3 MT. plus Glib.	
Age (years)	40.50 ± 8.18	53.65 ± 4.45 a	54.29 ± 3.47 b	53.70 ± 2.69 c	0.0001*
BMI (Kg/m^2)	23.16 ± 2.87	27.15 ± 3.66 a	25.98 ± 3.37 b	26.38 ± 3.89 c	0.0025*

Duration of diabetic (years)	---	---	3.09 ± 1.03	3.38 ± 1.08	0.4030
Duration of taking treatment(years)	---	---	2.14 ± 1.04	2.70 ± 1.11	0.1059
FPG(mmole/L)	4.67 ± 0.39	12.00 ± 2.34 a	6.96 ± 1.30 b d	7.70 ± 1.23 c e	0.0001*

*significant using ANOVA test at 0.05 level of significance.

a) indicate significant difference between control and Group1.

b) indicate significant difference between control and Group2.

c) indicate significant difference between control and Group3.

d) indicate significant difference between Group1 and Group2.

e) indicate significant difference between Group1 and Group3.

f) indicate significant difference between Group2 and Group3.

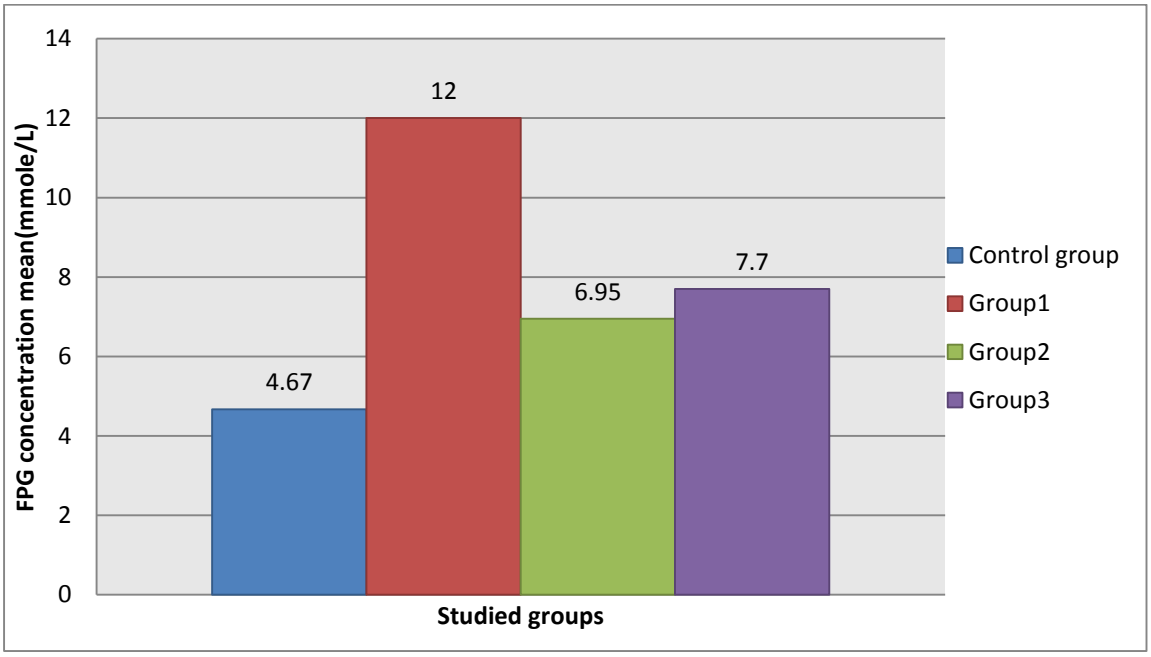


Figure (3.1):The level of FPG in three groups diabetic patients and control group.

3.2 Level of Glycated Hemoglobin (HbA1c%) in the Sera of Type 2 diabetic subjects and control

Table and figure (3.2) showed that the mean of glycated hemoglobin was found to be statistically significant ($p<0.05$) in group1,group2 and group3 when compared with control group. The HbA1c % level in group1 (newly diagnosed group) which do not take any type of anti-diabetic drugs has been statistically significant elevated as compared with those diabetic in group2 (with metformin therapy) and group3 (with metformin plus glibenclamide therapy). This result was in agreement with Krishna et al (2015) who have demonstrated that anti-diabetic drugs such as metformin and glibenclamide produced a significant decrease in HbA1c % levels compared to pre-treatment values [(132)]. On the other hand, there was a non significant difference between group2 and group3 this result is agree with Marwan (2013) who have demonstrated that there were a nonsignificant differences in HbA1c % levels between metformin treated group and those treated by a combination therapy of metformin plus glibenclamide [(131)].

Table(3.2) : Mean values of HbA1c% in three groups diabetic patients and control group.

Parameter	Mean ± SD				P-value
	Control group	Group1 Newly diagnosed	Group 2 MT.	Group 3 MT. plus Glib.	
HBA1c %	4.35 ± 0.63	9.39 ± 1.24 a	7.40 ± 1.84 b d	7.09 ± 1.02 c e	0.0001*

*significant using ANOVA test at 0.05 level of significance.

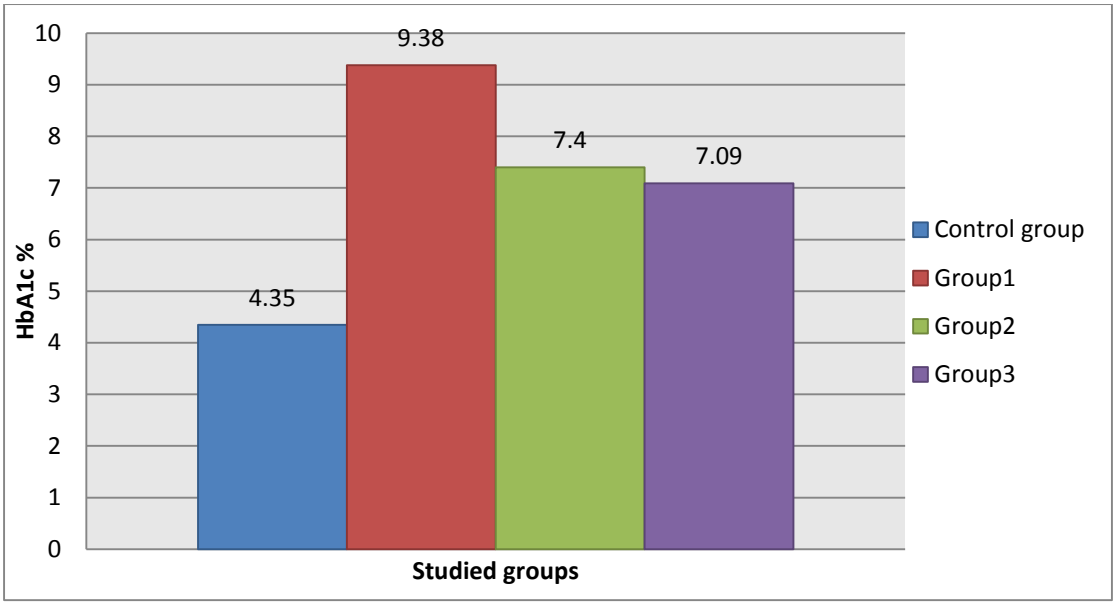


Figure (3.2):The level of HbA1c% in three groups diabetic patients and control group.

3.2 Lipid profile in the Sera of Type 2 diabetic subjects and control

3.3.1 Cholesterol

Table and figure (3.3) below showed that mean cholesterol was found to be statistically significant($p \leq 0.05$) in group1(newly diagnosed group) when compared with control group, This result was in agreement with Al-Naama et al (2010) who found that patients with T2DM have significantly higher serum concentrations of cholesterol [133] . Group1 showed a nonsignificant differences when compared with group2 and was a significant difference when compared with group3, so this result found that using metformin plus glibenclamide treatment showed a favorable effect on cholesterol than using metformin alone. A non-significant differences was observed in group2 (with metformin therapy) and group3 (with metformin plus glibenclamide therapy) when compared with control group This results in agreement with Reyadh et al (2012) who found that use of metformin / glibenclamide combination or metformin alone in the treatment of T2DM maintained cholesterol levels

closer to normal levels and in combination therapy serum TC level was lower than metformin used alone [134].

Table(3.3) : Mean values of serum cholesterol in three groups diabetic patients and control group.

Parameter	Mean ± SD				P-value
	Control group	Group1 Newly diagnosed	Group 2 MT.	Group 3 MT. plus Glib.	
Cholesterol (mmole/L)	4.06 ± 0.89	5.13 ± 1.15 a	4.45 ± 1.44	3.78 ± 1.29 e	0.0029*

*significant using ANOVA test at 0.05 level of significance.

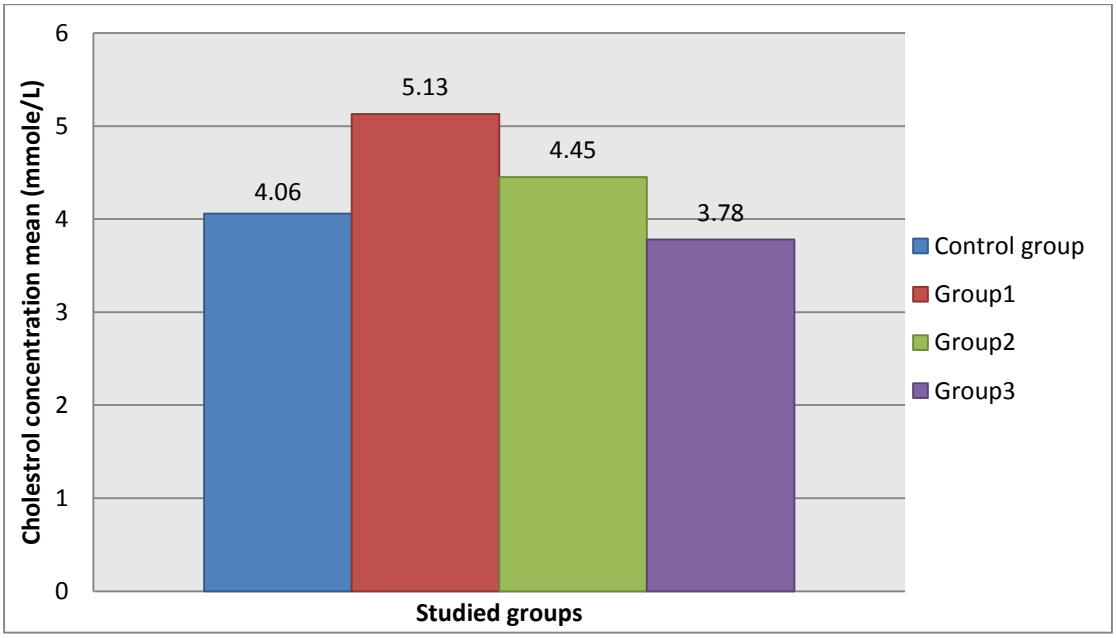


Figure (3.3): Serum cholesterol in three groups diabetic patients and control group.

3.3.2 Serum Triglyceride

Table and figure (3.4) below showed that mean triglyceride was found to be significantly elevated ($p \leq 0.05$) in group1(newly diagnosed group) when compared with control group this result in agreement with Attalah (2007) who found increase level of serum triglycerides in diabetic patients, which could be due to the increase of hepatic triglyceride synthesis[135].Group2(with metformin therapy) and group3(with metformin plus glibenclamide therapy) showed a non-significant differences when compared with group1 and control group these results were in agreement with Kassim (2011) who found that metformin and glibenclamide therapies produces a non-significant favorable effect on serum triglyceride [136].

Table(3.4) : Mean values of serum triglyceride in three groups diabetic patients and control group.

Parameter	Mean ± SD				P-value
	Control group	Group1 Newly diagnosed	Group 2 MT.	Group 3 MT. plus Glib.	
Triglyceride (mmole/L)	1.36 ± 0.45	2.09 ± 0.97 a	1.64 ± 0.71	1.81 ± 0.58	0.0111*

*significant using ANOVA test at 0.05 level of significance.

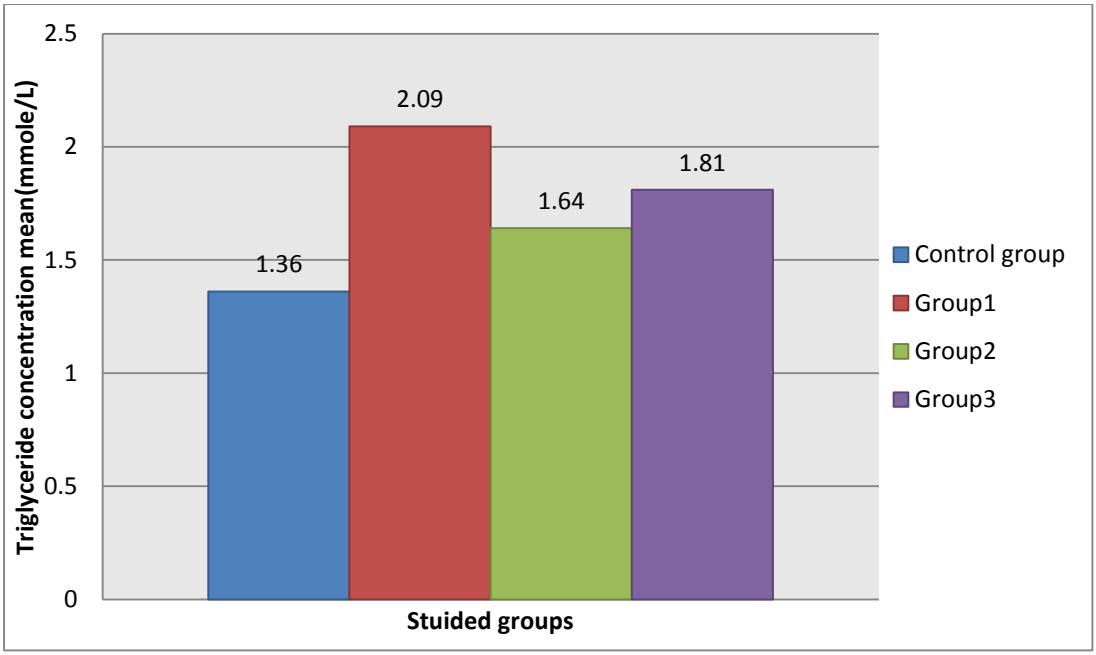


Figure (3.4):Serum Triglyceride in three groups diabetic patients and control group.

3.3.3 Serum High Density Lipid (HDL)

The results of serum high density lipoprotein for three groups diabetics and control group as shown in table and figure (3.5) revealed that mean HDL-C was found to be significantly different ($p \leq 0.05$) in diabetic patients in three groups when compared with control group. Type 2 diabetes is characterized by low HDL cholesterol (HDL-C) and HDL dysfunction [(137)]. The precise cause of the low HDL-C in type 2 diabetes is not known but may be the consequence of insulin resistance, augmented very low density lipoprotein production and increased activities of cholesteryl ester transfer protein and hepatic lipase. [(138)]. Group2(with metformin therapy) and group3(with metformin plus glibenclamide therapy) showed a non-significant differences when compared with group1 this result was in agreement with Cagatay et al (2011) who found that using metformin as a mono therapy or combination with glibenclamide in type 2 diabetics produce a non-significant effect on HDL-C [(139)].

Table(3.5) : Mean values of Serum HDL-C in three groups diabetic patients and control group.

Parameter	Mean ± SD				P-value
	Control group	Group1 Newly diagnosed	Group 2 MT.	Group 3 MT. plus Glib.	
HDL(mmole/L)	1.40 ± 0.31	1.08 ± 0.16 a	1.21 ± 0.19 b	1.11 ± 0.17 c	0.0001*

*significant using ANOVA test at 0.05 level of significance.

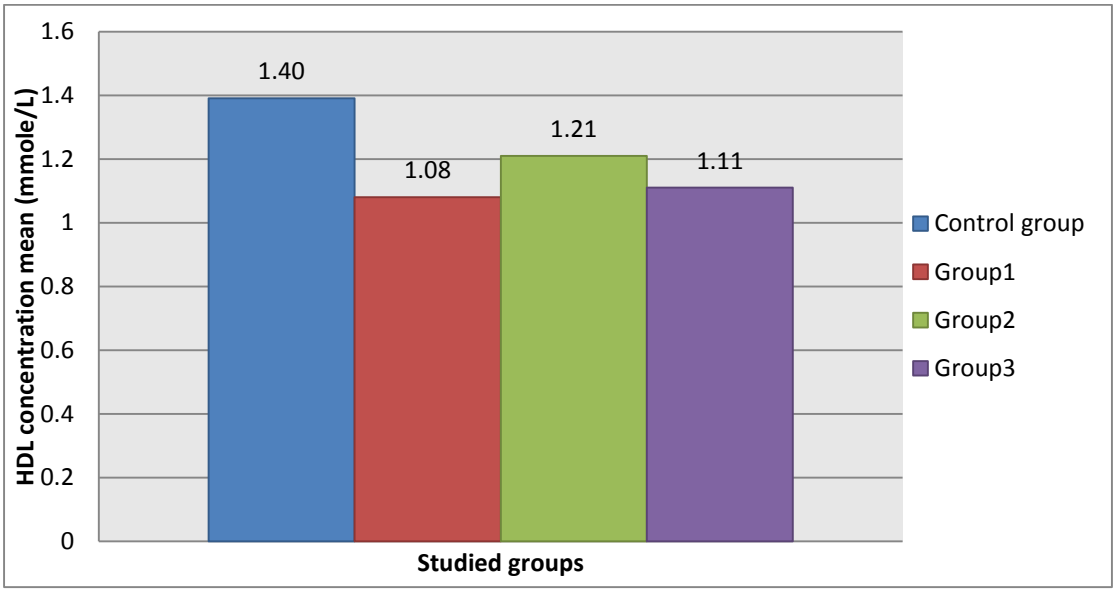


Figure (3.5): Serum HDL-C in three groups diabetic patients and control group.

3.3.4 Serum Low Density Lipid (LDL)

The results of serum low density lipoprotein for group1 and group2 diabetic patients was found to be significantly different ($p \leq 0.05$) when compared with control group. Singh and Kumar (2011) found that the level of LDL significantly higher in type 2 diabetics [(140)]. Increased elimination of lipids and apolipoproteins from VLDL particles results in the increased production of intermediate density lipoprotein (IDL) and LDL [(141)].Group1 showed a nonsignificant differences when compared with group2 and show a significant difference when compared with group3, so this result found that using metformin plus glibenclamide treatment showed a favorable effect on LDL than using metformin alone. Dailey et al (2002) found that combination therapy of metformin and glibenclamide shows a favorable effect on LDL-C levels and closer to that of non diabetic subjects [(142)].

Table (3.6) : Mean values of Serum LDL-C in three groups diabetic patients and control group.

Parameter	Mean ± SD				P-value
	Control group	Group1 Newly diagnosed	Group 2 MT.	Group 3 MT. Plus Glib.	
LDL(mmole/L)	1.80 ± 0.47	2.98 ± 0.89 a	2.57 ± 1.22 b	1.82 ± 1.27 e	0.0003*

*significant using ANOVA test at 0.05 level of significance.

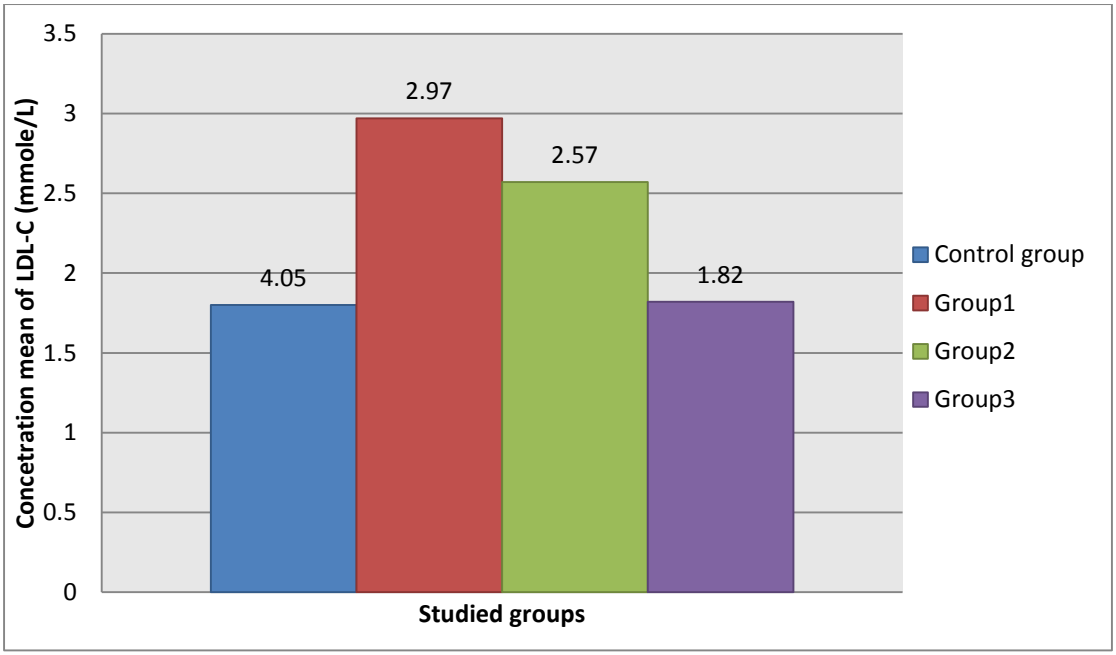


Figure (3.6): Serum LDL-C in three groups diabetic patients and control group.

3.3.5 Very Low Density Lipoprotein (VLDL)

The results of serum very low density lipoprotein for three groups diabetic and control group as shown in table and figure (3.7) revealed that mean VLDL-C in group1(newly diagnosed group) was found to be significantly elevated ($p \leq 0.05$) when compared with control group. This result was in agreement with Petrovic et al (2010) who showed a significant elevation in VLDL-C when compared diabetic patients with controls. This may be due to insulin resistance has striking effects on lipoprotein size and subclass particle concentrations for VLDL and that lead to increased hepatic secretion of VLDL-C in type 2 diabetic patients [(143)]. Comparing group2(with metformin therapy) and group3(with metformin plus glibenclamide therapy) with the control group showed a non significant differences this result was in agreement with Reyadh et al (2012) who found a nonsignificant difference between metformin and

metformin plus glibenclamide treated groups compared with control group [134].

Table(3.7) : Mean values of serum VLDL-C in three group diabetic patients and control group.

Parameter	Mean ± SD				P-value
	Control group	Group1 Newly diagnosed	Group 2 MT.	Group 3 MT. Plus Glib.	
VLDL (mmole/L)	0.618 ± 0.09	0.95 ± 0.19 a	0.745 ± 0.14	0.818 ± 0.12	0.0111*

*significant using ANOVA test at 0.05 level of significance.

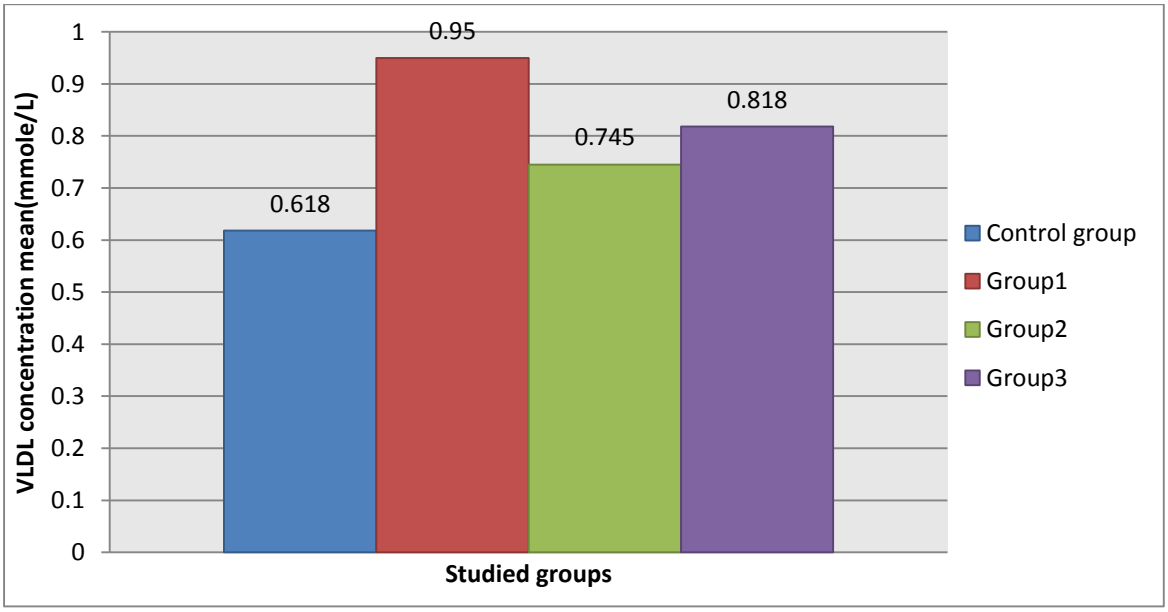


Figure (3.7): Serum VLDL-C in three groups diabetic patients and control group.

3.4 Leptin Hormone in Type 2 Diabetic Patients and NonDiabetic Counterparts

Table (3.8) showed that mean of leptin concentration and BMI was found to be elevated in diabetic patients as compared with control group, and the differences were statistically significant ($p \leq 0.05$). These results are similar to the observation of Fadhil et al (2011) who stated that serum leptin concentration and BMI was found to be elevated in diabetic patients as compared to non-diabetic subjects [(128)] figure (3.8) showed that mean of leptin concentration was found to be elevated in diabetic patients as compared with control group.The elevated leptin level in T2DM could be contributed to:

Leptin concentration is modulated by weight gain and loss in adult humans. Obesity is strongly associated with the development of T2DM. The adipose-derived hormone, leptin has been implicated in the regulation of body weight and energy homeostasis. Circulating leptin concentrations reflect the amount of adipose tissue in the body [(144,145)], and as we knowing the strong relationship between T2DM and obesity so the elevated leptin might be due to increased body mass index. In type 2 diabetic patients, insulin levels were elevated duo to insulin resistance and that may be stimulate leptin expression/ release [(146,147)].Wauters et al (2003) found that insulin secretion and the degree of insulin resistance contribute significantly to leptin levels [(148)].

Table (3.8) mean distribution of leptin in type 2 diabetic patients and nondiabetic counterparts.

Parameters	Mean ± SD		P-value
	Control group(N=20)	Diabetic group(N=64)	
Leptin (ng/ml)	5.73 ± 2.80	17.10 ± 8.30	0.0001*
BMI (Kg/m^2)	23.16 ± 2.87	26.52 ± 3.62	0.0003*

*Significant difference using student's t-test for comparing between two independent means at 0.05 level of significance

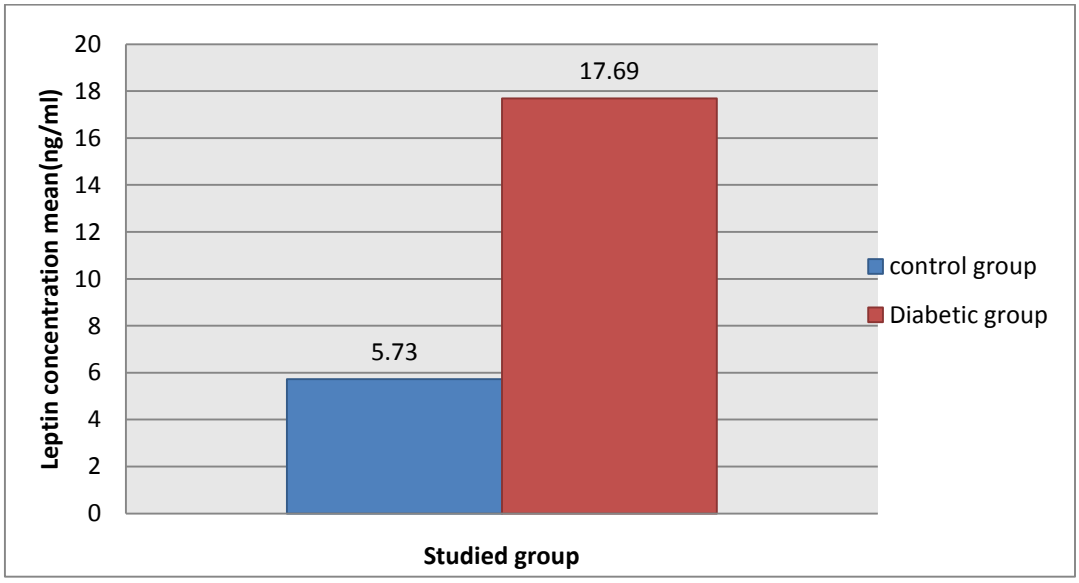


Figure (3.8): mean distribution of serum leptin concentration in type 2 diabetic patients and non diabetic counterparts.

Table and figure (3.9) showed the mean leptin concentration of the studied groups dividing them according to anti-diabetic therapy in group1 (newly diagnosed group) was found to be significantly elevated at ($P \leq 0.05$) as compared with group2 and group3 that treated with anti-diabetic drugs. Many studies have demonstrated that taking oral anti-diabetic drugs change the serum leptin concentration in type 2 diabetes [77,78]. In the present study, serum leptin concentration was significantly lowered in metformin treated group compared with metformin plus

glibenclamide treated group this indicate that metformin mono therapy affects the level of leptin more than metformin combination therapy. This result was in agreement with Taqua et al (2011) who reported that the use of metformin alone in diabetic patients for 90 days have significantly decreased serum leptin levels by about 40.6% [149]. Also, the treatment of diabetic subjects with different doses of metformin significantly decreases the serum levels of leptin after 3 months treatment compared to baseline value [150]. Metformin plus Glibenclamide treated group showed the highest level of leptin than metformin treated group. The increase in leptin levels was related to the change in insulin levels caused by glibenclamide. This observation is in agreement with Haffner et al (1991) who show that glibenclamide caused an increase in leptin level parallel to the change in insulin levels [151]. In 30 patients with type 2 diabetes mellitus, Bhattacharya et al (2008) showed a significant elevation in leptin level after treatment with glibenclamide for 10 weeks [152].Glibenclamide correct hyperglycemia by stimulating insulin secretion [153]. Insulin stimulates the secretion of leptin, which explains the elevated level of leptin by sulphonylureas (glibenclamide) [154].

Table(3.9) : Mean values of serum Leptin in three groups diabetic patients and control group.

Parameter	Mean ± SD				P-value
	Control group	Group1 Newly diagnosed	Group 2 MT.	Group 3 MT. Plus Glib.	
Leptin (ng/ml)	5.73 ± 2.80	21.67 ± 5.89 a	12.07 ± 6.77 b d	17.14 ± 9.31 c e f	0.0001*

*significant using ANOVA test at 0.05 level of significance.

a) indicate significant difference between control and Group1.

b) indicate significant difference between control and Group2.

c) indicate significant difference between control and Group3.

d) indicate significant difference between Group1 and Group2.

e) indicate significant difference between Group1 and Group3.

f) indicate significant difference between Group2 and Group3.

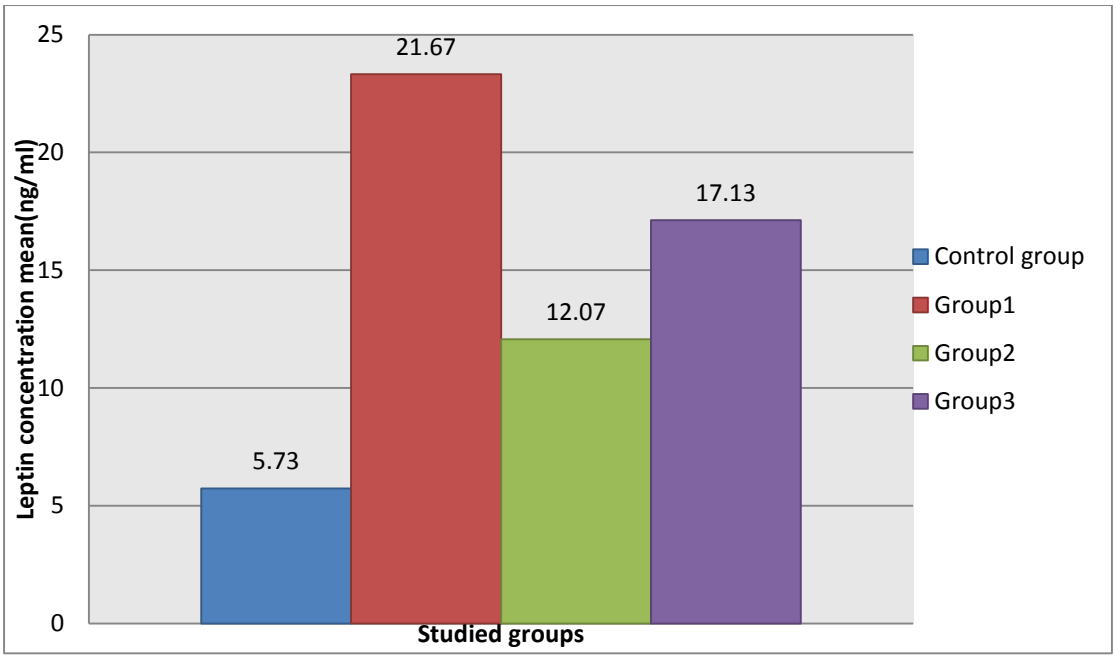


Figure (3.9): Serum Leptin concentration in three groups diabetic patients and control group.

3.5 Glucose-6-phosphatase Activity in Type 2 Diabetic Patients and NonDiabetic Counterparts

Table and figure (3.10) showed that mean G-6-Pase activity and FPG was found to be elevated in diabetic patients compared with control group, and the differences were statistically significant ($p \leq 0.05$).

A characteristic feature of type 2 diabetes is increased endogenous glucose production, largely due to increased hepatic glucose production (HGP) [155]. During fasting, hepatic gluconeogenesis is the primary source of endogenous glucose production and the major enzyme responsible for

the regulation of gluconeogenesis is glucose-6-phosphatase [156]. Common final pathway of glucose release involves the dephosphorylation of glucose via glucose-6-phosphatase (G-6-Pase) [157]. Hundal et al (2000) found that increased rate of glucose production in the diabetic subjects could be attributed to an increased rate of gluconeogenesis [158]. So our study found that G-6-Pase activity is increased, thereby leading to an increase in endogenous glucose production (EGP) in patients with type 2 diabetes and, therefore FPG will increase .This result agree with Clore J. et al (2000) who found that hepatic G-6-Pase activity determined from freshly isolated microsomes was significantly increased in the type 2 diabetic patients compared with the control subjects and rates of endogenous glucose production (EGP) were increased in the diabetic patients and were closely correlated with fasting plasma glucose [159].

Table (3.10) mean distribution of G-6-Pase and FPG in type 2 diabetic patients and nondiabetic counterparts.

Parameters	Mean ± SD		P-value
	Control group(N=20)	Diabetic group(N=64)	
G-6-Pase (U/ml)	30.90 ± 13.52	61.99 ± 43.41	0.0023*
FPG(mmole/L)	4.67 ± 0.39	9.00 ± 2.85	0.0001*

*Significant difference using student's t-test for comparing between two independent means at 0.05 level of significance

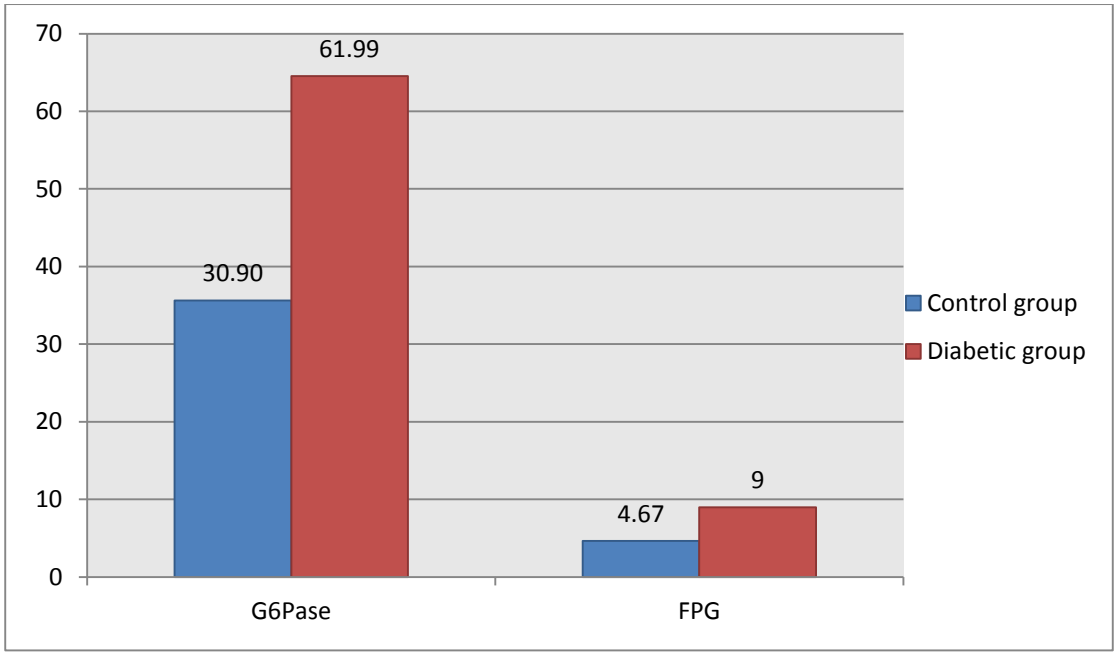


Figure (3.10): mean distribution of G-6-Pase activity and FPG concentration in type 2 diabetic patients and nondiabetic counterparts.

3.6 Glucose-6-phosphatase Activity in the Studied Groups Dividing them According to anti-diabetic therapy

Table and figure (3.11) showed that mean G-6-Pase activity and FPG concentration were found to be elevated in group1 (newly diagnosed group) when compared with group2 (with metformin therapy), group3 (with metformin plus glibenclamide therapy) and control group, and the differences was statistically significant ($p \leq 0.05$) .There were a nonsignificant difference between treated groups and control. Abnormally high liver glucose-6-phosphatase occurs in poorly controlled or untreated diabetes mellitus [160].Some previous studies showed that metformin therapy was normalized glucose-6-phosphatase activity in diabetic rats [161]. Kolawole and Akanji (2014) revealed that in vivo G-6-Pase activity of diabetic rats was significantly increased compared to that of non-diabetic control and treatment of diabetic rats with metformin for 28

days caused a significant decrease in the activity of G-6-Pase compared to diabetic control [162].Hundal et al (2000) found that metformin treatment in the diabetic human decreased rates of glucose production through a reduction in the rate of gluconeogenesis [158].This results revealed that using metformin as mono therapy or combination with glibenclamide reduce hepatic glucose production by reducing G-6-Pase activity leading to decrease FPG levels in type 2 diabetic patients.

Table(3.11) mean values of G-6-Pase activity and FPG in three groups diabetic patients and control group.

Parameter	Mean ± SD				P-value
	Control group	Group1 Newly diagnosed	Group 2 MT.	Group 3 MT. plus Glib.	
G-6-Pase(U/ml)	30.90 ± 13.52	113.95 ± 25.22 a	31.35 ± 13.47 d	34.44 ± 14.94 e	0.0001*
FPG(mmole/L)	4.67 ± 0.39	12.00 ± 2.34 a	6.96 ± 1.30 b d	7.70 ± 1.23 c e	0.0001*

*significant using ANOVA test at 0.05 level of significance.

a) indicate significant difference between control and Group1.

b) indicate significant difference between control and Group2.

c) indicate significant difference between control and Group3.

d) indicate significant difference between Group1 and Group2.

e) indicate significant difference between Group1 and Group3.

f) indicate significant difference between Group2 and Group3.

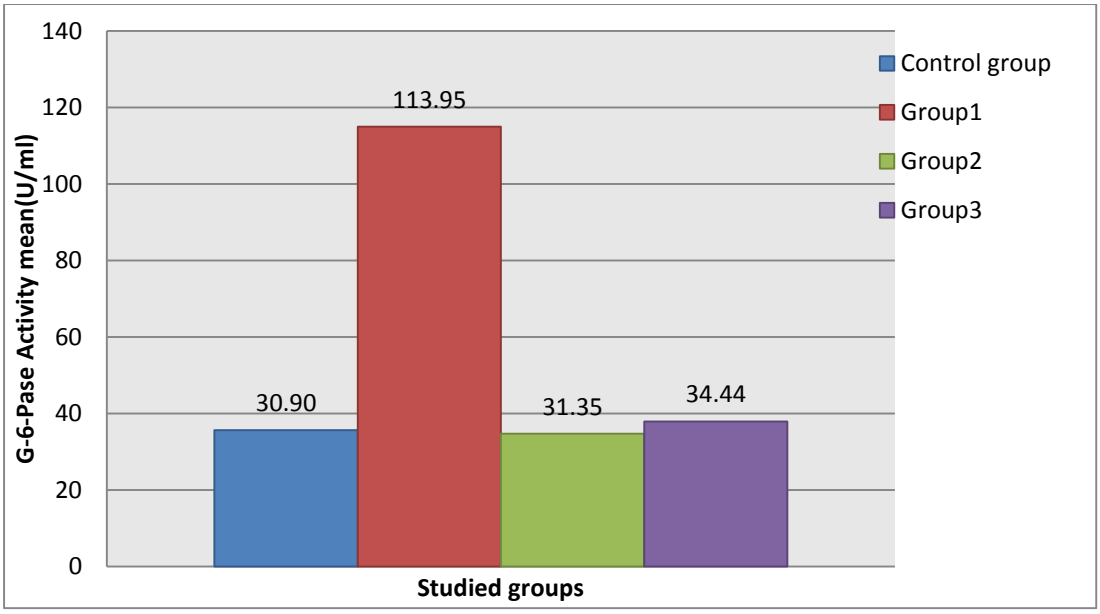


Figure (3.11) : levels of G-6-Pase activity in three groups diabetic patients and control group.

3.7 Correlation Between serum G-6-Pase Activity and other variable

3.7.1 G-6-Pase and Age:

There were no significant correlations observed in table (3.12) between G-6-Pase activity and age in diabetic patients (group1,group2 andgroup3)(r=0.171,p>0.05),(r=0.341,p>0.05),(r=0.412,p>0.05) respectively, and in control group (r=0.112, p >0.05).

3.7.2 G-6-Pase and BMI:

There were no significant correlations between G-6-Pase activity and BMI in diabetic patients (group1,group2 and group3) (r=0.251,p>0.05) , (r=-0.167,p>0.05), (r=0.054,p>0.05) respectively, and in control group ` (r=0.281, p >0.05).As shown in table (3.12).

3.7.3 G-6-Pase and Duration of Diabetes and duration of taking treatment:

There were no significant correlations between G-6-Pase activity and duration of diabetes in diabetic patients (group2 and group3) (r=-0.072,p>0.05) ,(r=-0.164,p>0.05) respectively. In duration of taking treatment there was a non-significant correlation with G-6-Pase in group2 (r=-0.122,p>0.05) and group3 (r=-0.267,p>0.05) as shown in table (3.12).

3.7.4 G-6-Pase and FPG:

There were positive significant correlations between G-6-Pase activity and FPG in diabetic patients (group1, group2 and group3) (r=0.658,p<0.01) ,(r=0.604,p<0.01), (r=0.600,p<0.05) respectively, and non-significant correlation in control group (r=0.421, p>0.05).As shown in table (3.12) and figures (3.12a)(3.12b)(3.12c)**.** The present study found that when G-6-Pase activity is increased in patients with type 2 diabetes the endogenous glucose production will increased and therefore FPG will increased. Clore J. et al (2000) found that hepatic G-6-Pase activity was significantly increased in the type 2 diabetic patients and rates of EGP were increased in the diabetic patients and were closely correlated with fasting plasma glucose **(159).**

3.7.5 G-6-Pase and HbA1c% :

There were positive significant correlations between G-6-Pase activity and HbA1c % in group1 (r=0.511,p<0.05) and group2(r=0.446,p<0.05) , and non significant correlations in group3 (r=0.430, p >0.05) and control group (r=0.322, p >0.05) .As shown in table (3.12) and figures (3.13a)(3.13b). This result found that increasing

G-6-Pase activity will increase the concentration of glucose in the blood and that will increase the long-term glycemic control(HbA1c%).

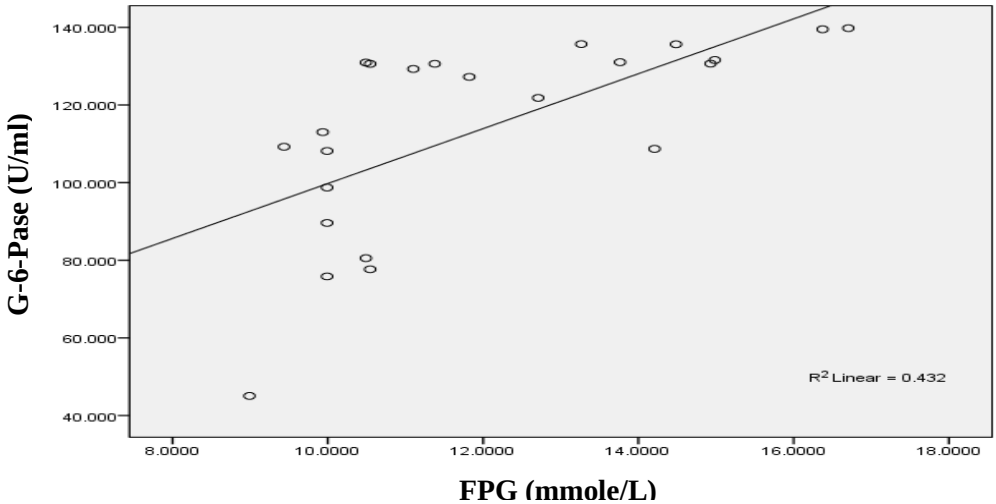
3.7.6 G-6-Pase and Leptin hormone:

There were no significant correlations between G-6-Pase activity and leptin in diabetic patients (group1,group2,group3) (r=0.009,p>0.05) ,(r=-0.153,p>0.05), (r=-0.026,p>0.05) respectively, and in control group (r=-0.171, p >0.05). As shown in table (3.12).

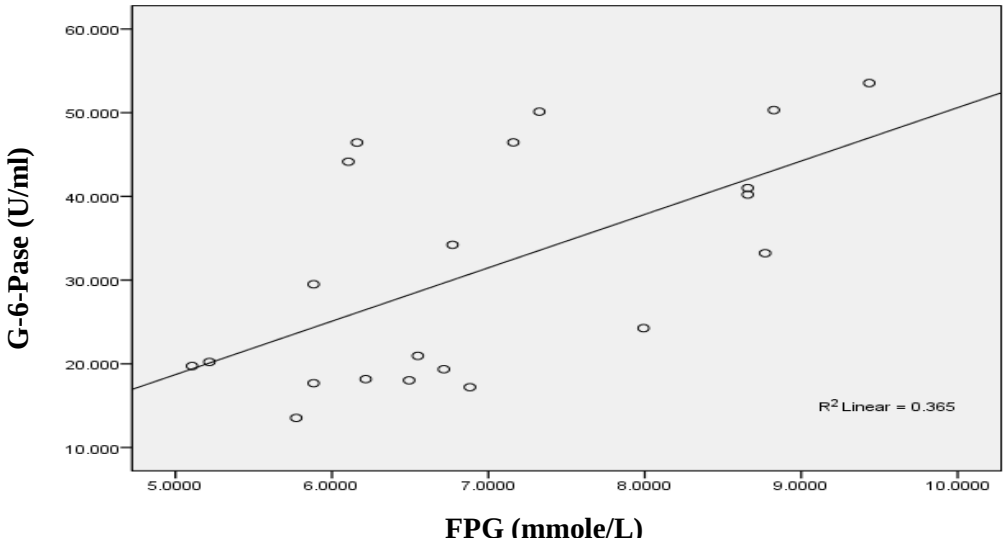
Table (3.12) Correlation coefficient between G-6-Pase and study parameters.

Parameters	Control group	Group1 Newly diagnosed	Group 2 MT.	Group 3 MT. Plus Glib.
Age(years)	0.112 NS	0.171 NS	0.341 NS	0.412 NS
BMI(kg/ m^2)	0.281 NS	0.251 NS	-0.167 NS	0.054 NS
Duration of diabetic(years)	---	---	-0.072 NS	0.164 NS
Duration of taking treatment(years)	---	---	-0.122 NS	-0.267 NS
FPG(mmole/L)	0.421 NS	0.658**	0.604**	0.600**
HBA1c %	0.322 NS	0.511*	0.466*	0.430 NS
Leptin(ng/ml)	-0.171 NS	0.009 NS	-0.153 NS	-0.026 NS

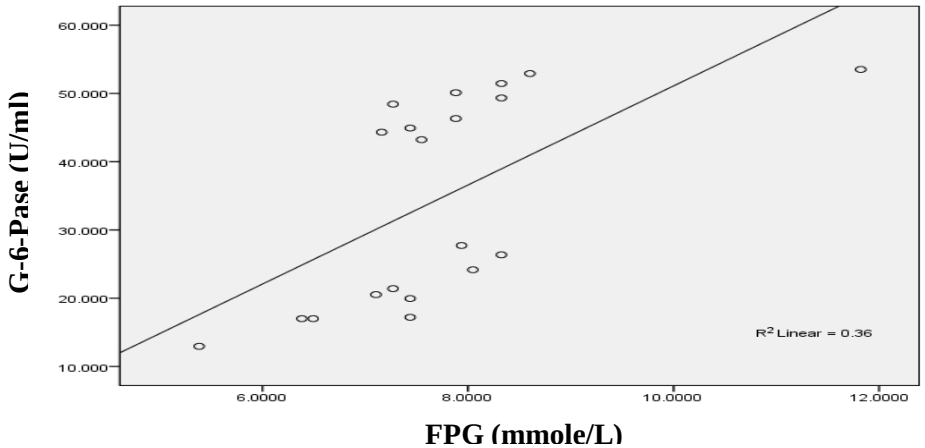
* (P≤0.05) ,**(P≤,0.01) , NS: Non-significant.



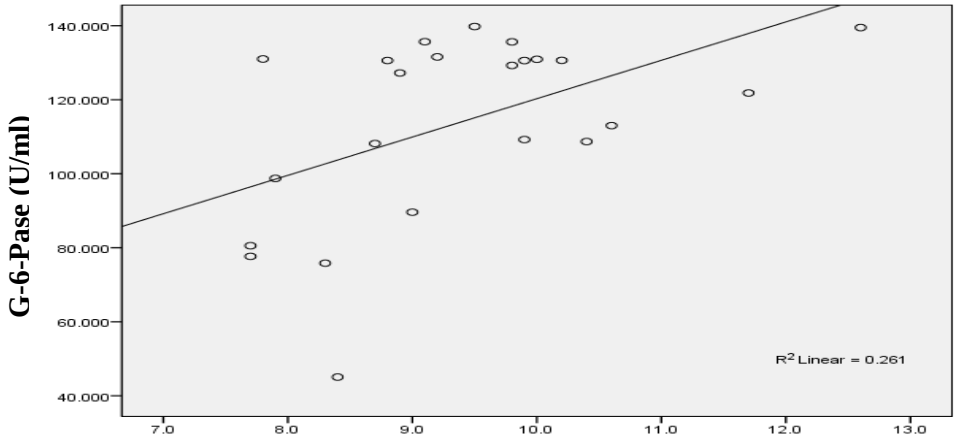
Figure(3.12a) : The correlation between G-6-Pase (U/ml) and FPG (mmole/L) in group1.



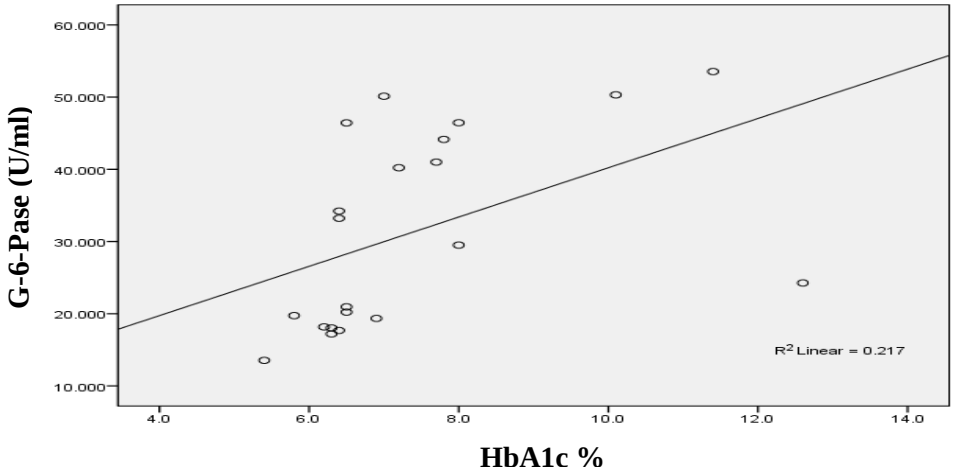
Figure(3.12b) : The correlation between G-6-Pase (U/ml) and FPG (mmole/L) in group2.



Figure(3.12c) : The correlation between G-6-Pase (U/ml) and FPG (mmole/L) in group3.



Figure(3.13a) : The correlation between G-6-Pase (U/ml) and HbA1c % in group1.



Figure(3.13b) : The correlation between G6Pase (U/ml) and HbA1c % in group2.

3.8 Correlation Between serum Leptin hormone and other variable

3.8.1 Leptin and Age:

There were no significant correlations observed in table (3.13) between leptin hormone and age in diabetic patients (group1,group2, group3) (r=-0.015,p>0.05), (r=-0.134,p>0.05), (r=-0.304,p>0.05) respectively, and in control group (r=-0.302, p >0.05).

3.8.2 Leptin and BMI:

There were positive significant correlations between leptin hormone and BMI in diabetic patients(group1,group2 ,group3) (r=0.465,p<0.05) ,(r=0.445,p<0.05), (r=0.531,p<0.05) respectively, and non-significant correlation in control group (r=0.295, p >0.05).As shown in table (3.13) and figures (3.14a)(3.14b)(3.14c). In the present study, a significant positive correlation was found between serum leptin level and BMI, which was in agreement with many other studies (Pandey et al 2015 , Mahmood et al 2013 , Buyukbese et al 2004) [163,164,165]. leptin is secreted by the adipose tissue in proportion to adipose mass therefore its circulating levels increase with weight gain and decrease with weight loss [166].

3.8.3 Leptin and Duration of Diabetes and duration of taking treatment :

There were no significant correlations between leptin hormone and duration of diabetes in group2 (r=0.317,p>0.05) and group3 (r=0.054,p>0.05) . In duration of taking treatment there was positive significant correlation between leptin hormone and duration of taking

treatment in group2 (r=0.449,p<0.05) and non-significant correlation (r=0.075,p>0.05) in group3 as shown in table (3.13).

3.8.4 Leptin and FPG:

There were no significant correlations between leptin hormone and FPG in diabetic patients(group1,group2 and group3) (r=0.285,p>0.05) , (r=-0.394, p <0.05) ,(r=-0.174,p>0.05) respectively ,and in control group (r=-0.262, p <0.05) as shown in table (3.13). This results were in agreement with Chen C. et al (2003) and Mahmood et al (2013) who found nonsignificant correlations between leptin hormone and FPG in diabetic patients [167,164].

3.8.5 Leptin and HbA1c% :

There were positive significant correlation between leptin hormone and HbA1c % in group1 (r=0.442, p <0.05) .And a non significant correlation in group2,group3 and control group (r=-0.243,p>0.05) ,(r=-0.013,p>0.05), (r=-0.124, p <0.05) respectively as shown in table (3.13) and figure (3.15).

3.8.6 Leptin and Serum Lipid Profile:

There were no significant correlations between leptin hormone and cholesterol, triglyceride, HDL-C and LDL-C in diabetic patients groups and control group as shown in Table(3.13).This result agrees with Hsu C. et al (2008) who found a non-significant correlations between leptin and lipoprotein in patients having Type 2 diabetes for more than one year and taking anti-diabetic therapy for more than 6 months [168].

Table (3.13) Correlation coefficient between Leptin and study parameters.

Parameters	Control Group	Group1 Newly diagnosed	Group 2 MT.	Group 3 MT. Plus Glib.
Age (years)	-0.302 NS	-0.015 NS	-0.134 NS	-0.304 NS
BMI (kg/ m^2)	0.295 NS	0.465 *	0.445 *	0.531 *
Duration of diabetic(years)	---	---	0.317 NS	0.054 NS
Duration of taking treatment(years)	---	---	0.449 *	0.075 NS
FPG(mmole/L)	-0.262 NS	0.285 NS	-0.394 NS	-0.174 NS
HBA1c %	-0.124 NS	0.442*	-0.243 NS	0.013 NS
G-6-Pase(U/ml)	-0.171 NS	0.009 NS	-0.153 NS	-0.026 NS
Cholesterol (mmole/L)	-0.082 NS	-0.184 NS	0.149 NS	0.358 NS
Triglyceride (mmole/L)	0.213 NS	0.024NS	0.273 NS	-0.131 NS
HDL(mmole/L)	-0.391 NS	-0.106 NS	0.085 NS	0.370 NS
LDL(mmole/L)	-0.035 NS	0.077 NS	0.142 NS	0.253 NS

* ($P \leq 0.05$),**($P \leq 0.01$) , NS: Non-significant.

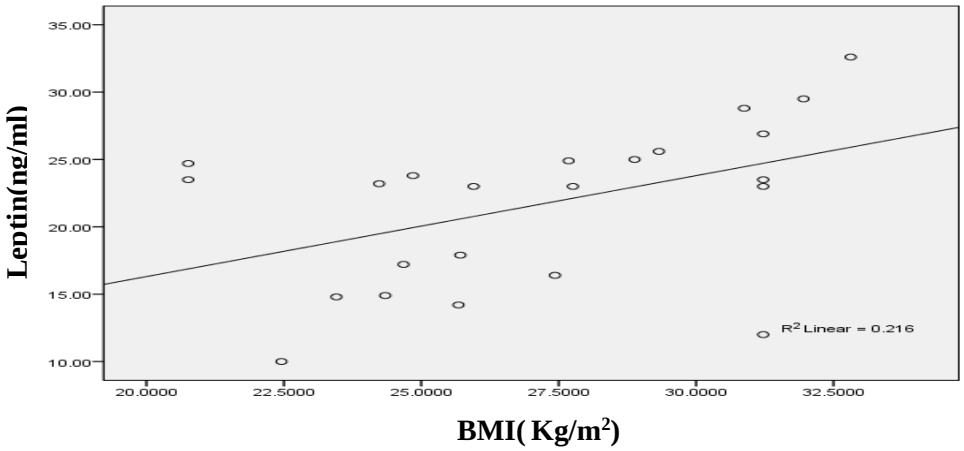
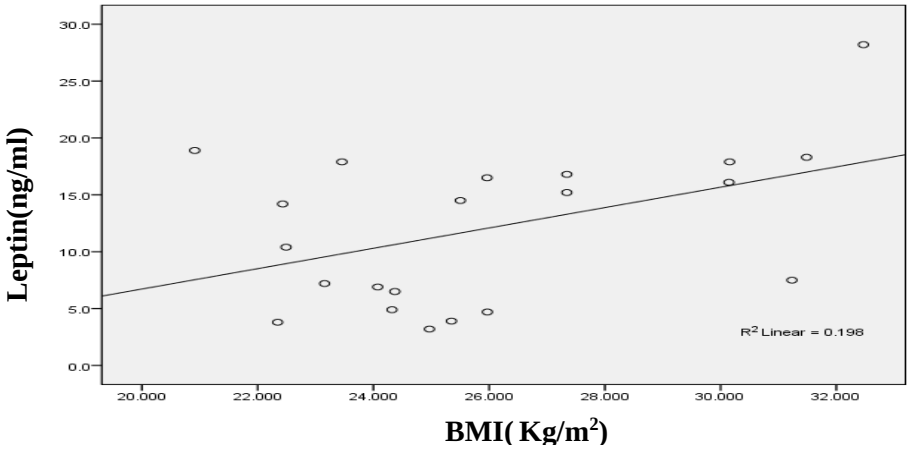


Figure (3.14a) : The correlation between leptin (ng/ml) and BMI (Kg/m^2) in group1.



Figure(3.14b) : The correlation between leptin (ng/ml) and BMI (Kg/m2) in group2.

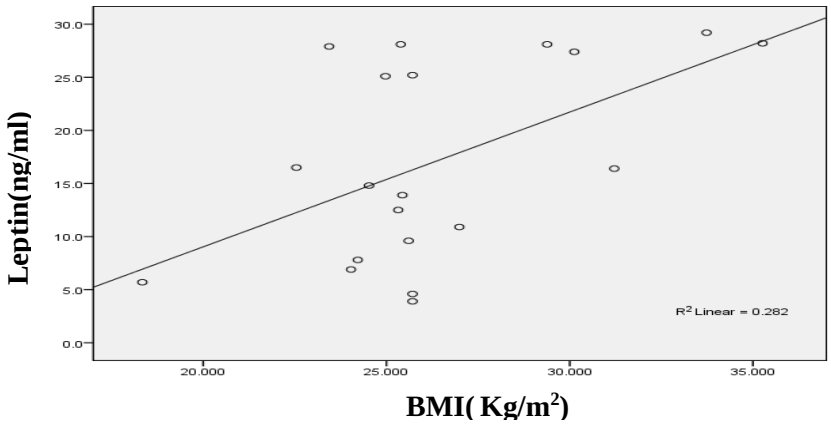


Figure (3.14c) : The correlation between leptin (ng/ml) and BMI (Kg/m2) in group3.

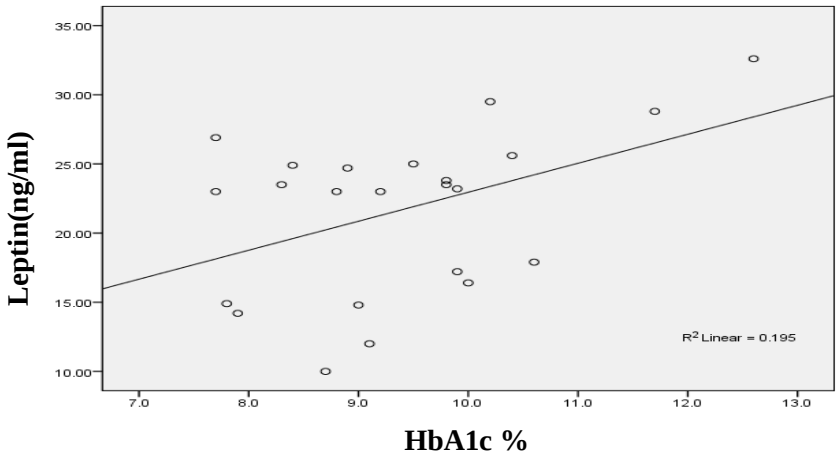


Figure (3.15) : The correlation between leptin (ng/ml) and HbA1c % in group1.

3.9 Binding studies of Glucose-6-Phosphatase with Biotin Antibody in the sera of Patients with T2DM:

The factors that affect the binding between Glucose- 6-Phosphatase and biotin antibody in the sera of Group1 (newly diagnosed), group2 (with metformin therapy),group3 (with metformin plus glibenclamide) and control group were evaluated, the result revealed:

3.9.1 The Effect of Different Concentrations of biotin Antibody on the binding with sera G-6-Pase:

The binding of the antigen to its antibody is an equilibrium reaction. This experiment was carried out to determine the appropriate concentration of biotin antibody that gives the highest Antibody-Antigen (Ag-Ab) complex formation:

Fixed amounts of the sera of patients with type 2 DM and control group (50µls) were incubated with increasing volume of biotin antibody (15, 25, 50, 75 and 100µls) for (1hour) at 37 ^{o}C. The results are illustrated in Figure (3.16 a).

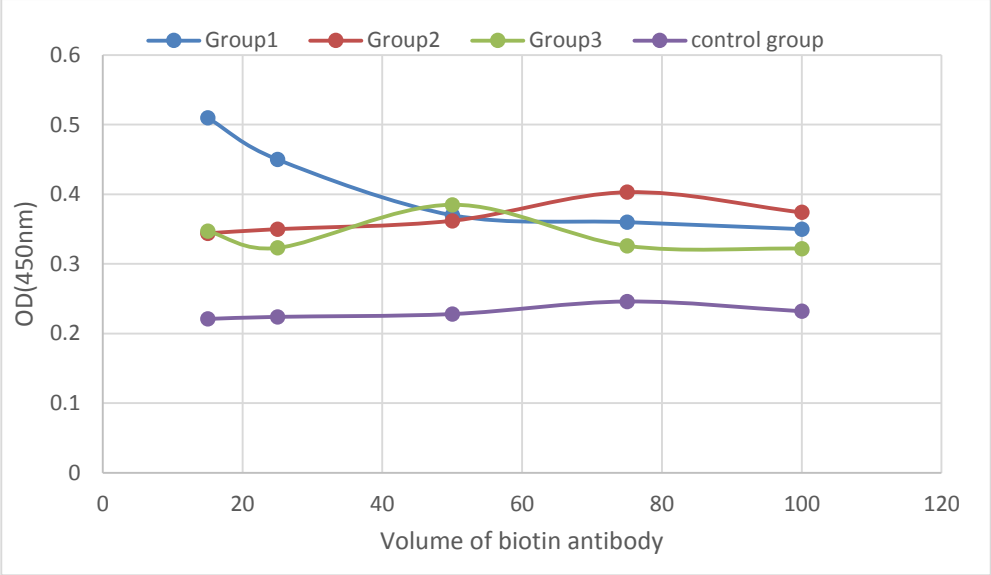


Figure (3.16 a): **Effect of Different volumes of biotin antibody on the Binding with G-6-Pase**.

Group1 have the high level of G-6-Pase while group2 and group3 have slightly higher or normal level of G-6-Pase. It is obvious that the OD of the binding of group2, group3, and control group was risen by increasing the amount of biotin antibody and then was saturated at (75µls) in group2 and control group,(50 µls) in group3, while in group1 appeared to be saturated at (15 µls).

3.9.2 The Effect of Different Concentrations of sera G-6-Pase on the binding with biotin antibody:

One of the factors that affect the binding of antibody-antigen reaction is the concentration of the enzyme in sera(Antigen).Increasing concentrations of G-6-Pase in increasing volumes of sera containing (15, 25, 50, 75 and 100µls) were incubated with a fixed amount of biotin antibody(15 µls of group1),(75 µls of group2 and control group),(50 µls of group3) for 1 hours at 37 ^{o}C.Group1 have the high level of G-6-Pase

while group2 and group3 have slightly higher or normal level of G-6-Pase. Therefore antibody- combining sites saturated at (15µls) group1,(50 µls) in group2 and control group,(25 µls)in group3. Figure (3.16 b) shows the maximal binding of the antibody with G-6-Pase in the four different groups.

If the number of antibodies- combining sites is significantly greater than the antigen binding sites [Ab] >>>[Ag], then enzyme binding sites are quickly saturated by the antibody before the cross- linking can occur and the formation of small Ab - Ag complexes of the composition Ab - Ag result. When antibody is in moderate excess [Ab] > [Ag], the probability of cross- linking of enzyme by antibody is more likely to occur, a large complex formation is favored. When enzyme is in great excess, large complexes would be $(Ag)_2$ Ab **(169).**

The results obtained in these experiments were used in all the subsequent experiments**.**

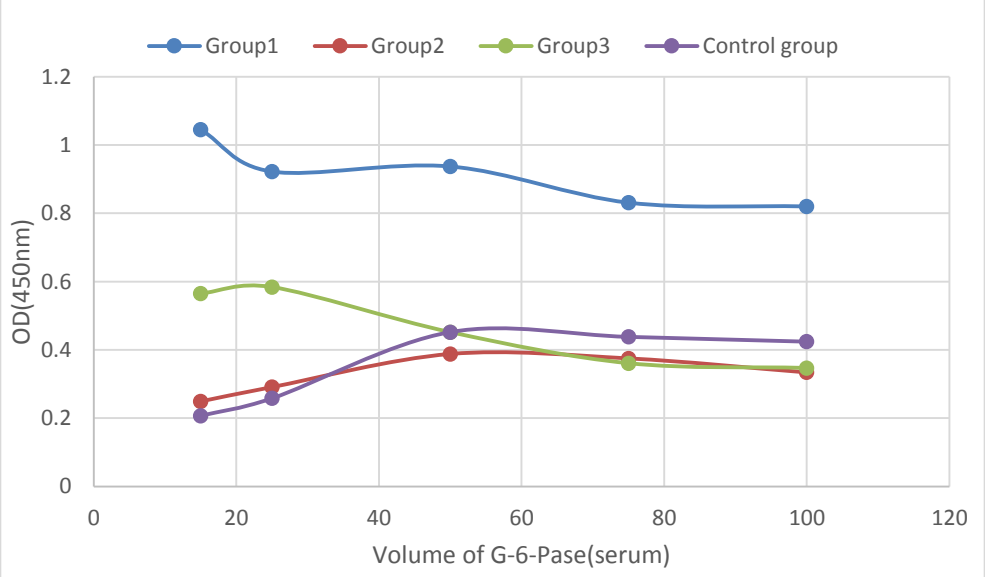


Figure (3.16 b): **Effect of Different volumes of G-6-Pase on the Binding with biotin antibody.**

2.9.3 The Effect of Temperature on the Binding of sera G-6-Pase with biotin antibody:

Figure (3.16 c) shows the result of this analysis. It shows that in group1 A maximal binding was obtainment at 45 ^{o}C. In group2, group3 and control group a maximal binding was obtainment at 37 ^{o}C. In all groups, OD increased as temperature expanded until it reached a maximum value then dropped gradually after that.

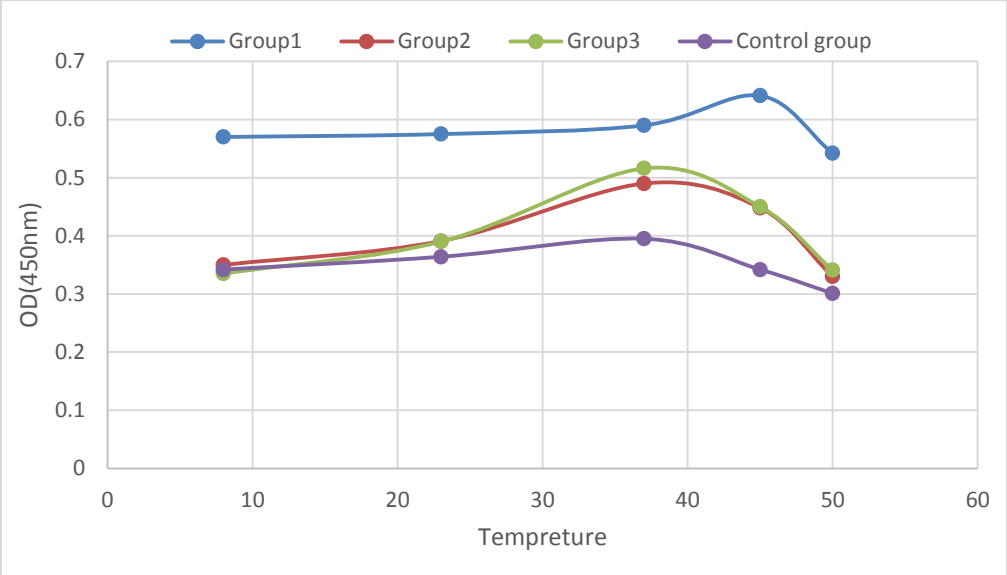


Figure (3.16 c): Effect of different temperature on the binding of G-6-Pase with biotin antibody.

It seems that the loss of the binding activity when temperature was raised may be due to the fact that dissociation rates show a greater increase with temperature than association rates, giving a higher affinity constant at a lower temperature, rise in temperature also leads to thermal denaturation of protein enzyme molecule [(170)] or it may be due to the irreversible dissociation of the Antibody-Antigen complex [(169)], The results obtained in these experiments were used in all the subsequent experiments.

2.9.4 The Effect of Incubation Time on the Binding of sera G-6-Pase with biotin antibody:

The experiment was carried out at different time intervals (15-120 minute). As shown in Figure (3.16 d) In all groups, OD increased when incubation time expanded, the optimal binding occurred within 90 minutes at 45 ^{o}C in group1, 60 minutes at 37 ^{o}C in group2, group3, and control group.

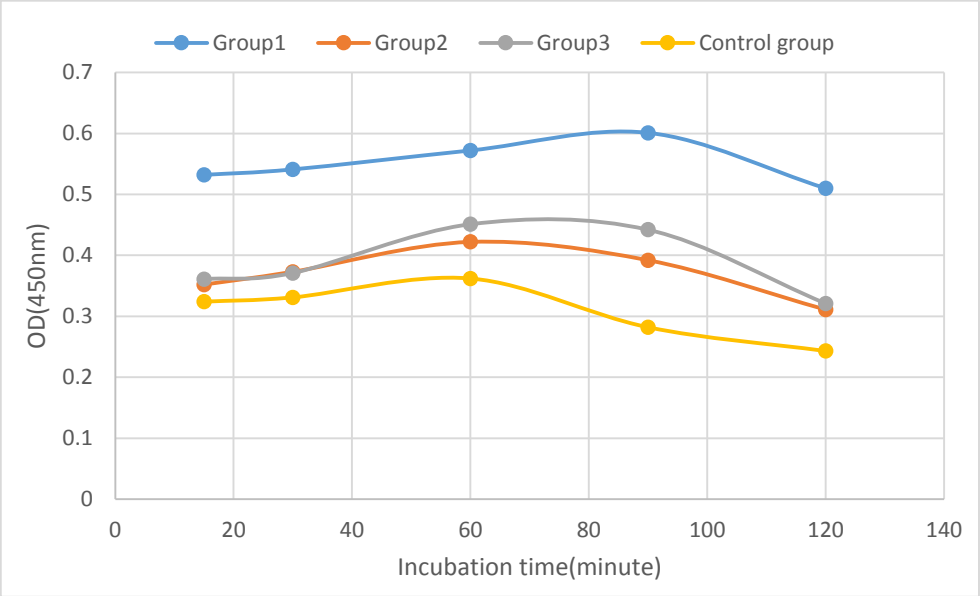


Figure (3.16 d): Effect of different incubation time on the binding of G-6-Pase with biotin antibody.

It seems that increased the binding activity when incubation time was raised may be due to the fact that sensitivity increased with a longer incubation time [(171)].Group1 have the higher level of G-6-Pase so it needs more time to give maximum binding allowing the enzyme to be completely saturated with the antibody until it reaches to optimum time.

Conclusions

And

Recommendation

4.1 Conclusions:

From this study, the following conclusions can be drawn:

In part one of the study

1. The levels of G-6-Pase activity and leptin hormone are found to be significantly higher in diabetic patients compared with healthy control group and found to be significantly higher in a newly diagnosed group when compared with metformin group, metformin plus glibenclamide group and control.

2. Using metformin as mono therapy or combination with glibenclamide can reduce FPG and HbA1c% levels in type 2 diabetic patients.

3. Metformin monotherapy reduced leptin levels more than metformin combination with glibenclamide in type 2 diabetic patients.

4. G-6-Pase activity and leptin hormone can be used as an indicator for the choice of treatment in those diabetic patients.

5. Metformin alone produce a non-significant favorable effect on all lipids profile parameters while metformin plus glibenclamide showed a significant reduction in TC and LDL-C.

In part two of the study

The effect of various factors on the binding study like [Antibody concentration, enzyme concentration, temperature and incubation time] were found different values for three groups diabetics and control as shown in table below .

Factors	control group	Group1	Group2	Group3
Concentration of biotin	75 µL	15 µL	75 µL	50 µL
Concentration of enzyme G-6-Pase	50 µL	15 µL	50 µL	25 µL
Temperature	37 ^{o}C	45 ^{o}C	37 ^{o}C	37 ^{o}C
Incubation time	60 mints	90 mints	60 mints	60 mints

4.2 Recommendations

1. Glucose-6-Phosphatase activity and leptin hormone can be used as an indicator for the choice of treatment in type 2 diabetic patients.

2. Study Glucose-6-Phosphatase activity in type1 diabetic subjects.

3. Study the gene expression of Glucose-6-Phosphatase enzyme in type 2 diabetic patients.

4. Evaluation of other clinical markers such as adiponectin, resistin, visfatin and others that are secreted from the adipose tissue and their correlations with T2DM.

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الخلاصة

خلفية البحث :

داء السكري من النوع الثاني هو مرض مزمن يتميز بارتفاع نسبه السكر في الدم حتى خلال فتره الصيام ويرجع ذلك بشكل كبير إلى زيادة أنتاج السكر داخل الجسم. والجلوكوز-٦-فوسفاتيز هو الإنزيم الذي يحفز الخطوة النهائية من تحلل الغليكوجين واستحداث السكر في الكبد وان الدور الكلاسيكي للجلوكوز-٦- فوسفاتيز في الكبد هو إنتاج الجلوكوز لإطلاقه في الدم.هذه العلاقه بين مرض السكري من النوع الثاني وانزيم الجلوكوز-٦- فوسفاتيز يجعل الانزيم هدفا للعلاجات للسيطره على مستويات السكر في الدم ان تثبيط الانزيم يؤدي الى تقليل تحرر الكلوكوز في الدم .تهدف هذه الدراسة الى تقييم آثار خافضات السكر الفموية (الميتفورمين وميتفورمين مع الجلايبينكلامايد) على نشاط أنزيم جلوكوز-٦- فوسفاتيز، تركيز هرمون اللبتين، سكر بلازما الدم الصائم (FPG)، نسبة السكر التراكمي HbA1c ٪ ومستوى الدهون في مرضى السكري من النوع الثاني ودراسة العوامل التي تؤثر على الارتباط بين الجلوكوز- ٦-فوسفاتيز والأجسام المضادة في جميع مرضى السكري ومجموعه السيطرة.

العينات والمواد وطرق العمل :

تضمنت هذه الدراسة (٨٤) عينة ،تراوحت أعمارهم بين (٤٠ - ٥٤) سنة .تم اختيار ٢٠ عينة لمجموعه السيطره و٦٤ عينة مرضى مصابين بداء السكري من النوع الثاني ، تم تقسيمهم إلى ثلاث مجاميع اعتمادا على نوع العلاج المستخدم : (٢٣) مريضا تم تشخيصهم حديثا من دون اخذ أي نوع من العلاج ، (٢٠) مريضا تمت معالجتهم بعقار الميتفورمين، (٢١) مريضا تمت معالجتهم بعقار الميتفورمين مع الجلايبينكلامايد ، في الجزء الأول من هذه الدراسة تم تعيين مستوى فعاليه أنزيم الجلوكوز- ٦-فوسفاتيز (G-6-Pase) ومستوى تركيز هرمون اليبتين كان التقييم بواسطة فحص (ELISA). وقيمت أيضا فترة الإصابة بالمرض ،فتره اعتماد العلاج ، ،سكر بلازما الدم الصيامي (FPG) ،نسبه السكر التراكمي (HbA1c%) ، دالة كتلة الجسم (BMI) و نسبه الدهون في مصل الدم. في الجزء الثاني من هذه الدراسة تم توصيف ارتباط الضد مع المستضد (biotin مع G6Pase) في مصل الأصحاء والمرضى وتمت دراسة العوامل المختلفة التي تؤثر على هذا الربط مثل: تركيز الأجسام المضادة، تركيز المستضد، درجة الحرارة وفترة الحضانة.

النتائج :

كانت النتائج المستحصلة من هذه الدراسة هي :

الجزء الاول :

- ❖ توجد زيادة معنوية واضحة في مستويات فعاليه انزيم G-6-Pase وهرمون اليبتين في أمصال المصابين بالسكري عند مقارنتهم مع مجموعه السيطرة ($P<0.05$).
- ❖ توجد زيادة معنوية واضحة في مستويات فعاليه انزيم G-6-Pase وهرمون اليبتين للمجموعة التي تم تشخيصها حديثا عند مقارنتهم مع ألمجموعه التي تمت معالجتهم بلميتفورمين ،ألمجموعه التي تمت معالجتهم بميتفورمين مع الجلاييبينكلامايد ومجموعه السيطرة ($P<0.05$).
- ❖ يوجد ارتباط ($P<0.05$) بين مستويات فعاليه الإنزيم (G-6-Pase) وسكر بلازما دم الصيامي (FPG) في كل مجاميع المرضى المصابين بلسكري من النوع الثاني وأيضا هناك ارتباط بين فعاليه هذا الإنزيم مع نسبه السكر التراكمي(HbA1c%) في المجوعه التي تم تشخيصها حديثا وألمجموعه التي تمت معالجتهم بلميتفورمين
- ❖ لا يوجد ارتباط ($P>0.05$) بين مستويات تركيز هرمون اليبتين مع فعاليه أنزيم G-6-Pase بينما يوجد ارتباط ($P<0.05$) بين مستوى تركيز اليبتين و داله كتله الجسم BMI في كل مجاميع المرضى المصابين بلسكري من النوع الثاني.

الجزء الثاني :

دراسة الارتباط بين الضد مع المستضد باستخدام فحص ELISA كشفت بأن العوامل المثلى التي تؤثر على الارتباط هي: تركيز البيوتين ، تركيز انزيم G-6-Pase ، درجه الحرارة وفتره الحضانة .

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جامعة النهرين
كلية العلوم
قسم الكيمياء

تأثير العقاقير المضادة لمرض السكري على فعالية أنزيم الجلوكوز-٦- فوسفاتيز في المرضى المصابين بداء السكري من النوع الثاني

رسالة

مقدمة إلى كليه العلوم/ جامعة النهرين

كجزء من متطلبات نيل درجه الماجستير في علوم الكيمياء.

من قبل

زينب صالح حلاب

بكالوريوس ٢٠١٣

بأشراف

الاستاذ المساعد

الدكتور علاء حسين جواد

جمادي الاخر
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