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EFFICACY AND SAFTEY OF METFORMIN AND COMBINATION THERAPY OF INSULIN AND METFORMIN IN TYPE 2 DIABETIC PATIENTS

BY

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بسم الله الرحمن الرحيم

إِنَّا فَتَحْنَا لَكَ فَتْحًا مُّبِينًا (1) لِيَغْفِرَ لَكَ اللَّهُ مَا تَقَدَّمَ مِن ذَنبِكَ وَمَا تَأَخَّرَ وَيُتِمَّ نِعْمَتَهُ عَلَيْكَ وَيَهْدِيَكَ صِرَاطًا مُّسْتَقِيمًا (2)وَيَنصُرَكَ اللَّهُ نَصْرًا عَزِيزًا (3)

صدق الله العظيم

DEDICATION

This work is dedicated to all members of my family my daughters& my *belOved* sister Eman

&my supervisor &my friends

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CERTIFICAE

I hereby certify that the work embodied in this thesis is the result of my own investigations except where reference has been made to published literature.

Signed…….candidate.

Signed…….supervisor of Studies.

DECLARATION

I here declare that this work has not already been accepted for any degree and is not been concurrently submitted in candidature for any degree.

Signed…….candidate.

Abbreviations

AACE	**American Association of clinical Endocrinologists**
ACE	**American college of Endocrinology.**
ADA	**American diabetic association.**
AI	**Atherogenic index**
ALP	**Alkaline phosphatase.**
ALT	**Alanine transferase**
AMP	**Adenosine mono phosphate**
AMPK	**Adenosine mono phosphate activated protein kinase**
AST	**Aspartate aminotransferase**
BMI	**Body mass index**
CDC	**Center for Disease Control and Prevention**
CT	**Creatinine**
CVD	**Cardiovascular disease.**
DKA	**Diabetic ketoacidosis**
DPP	**Diabetic prevention program**
DPP-4	**Dipeptidyl-peptidase-4**
EASD	**European Association For The Study of Diabetes.**
FBG	**Fasting blood glucose**

FDA	**Food Drug Administration**
GDM	**Gestational diabetes mellitus**
GLP-1	**Glucagon-like peptide -1**
GTUT4	**Glucose Transportr-4**
HBA1c	**Glycosylated hemoglobin**
HDL –C	**High density lipoprotein cholesterol**
HHS	**Hyperosmolar hyperglycemia state**
HONK	**Hyperosmolar non ketotic state**
HPLC	**High –performance liquid chromatography**
ID	**International Dollar**
IDDM	**Insulin-dependent diabetes mellitus**
IDF	**International diabetes federation**
IEC	**International expert committee**
IU	**International Unit**
IFG	**Impaired fasting glucose.**
IGT	**Impaired glucose tolerance**
INGA	**Islet Neogensis Associated Protein**
IR	**Insulin resistant**

LADA	**Latent autoimmune diabetes of adult**
LDL-C	**Low density lipoprotein cholesterol**
LFT	**Liver function test**
NAFLD	**Non alcoholic fatty liver disease**
NCDs	**Non communicable diseases.**
NHANE	**National health and nutrition examination**
NIDDM	**Non Insulin dependent diabetes mellitus**
OGTT	**Oral glucose tolerance test**
PCOS	**Polycystic ovarian syndrome**
PPARs	**Peroxizome proliferated activated receptors**
PPBG	**Post prandial blood glucose**
RDS	**Respiratory distress syndrome**
RFT	**Renal function test**
SEM	**Standard error of mean**
TC	**Total cholesterol**
TG	**Triglyceride**

TZDs	**Thiazolidinediones**
UIC	**University of iilons at Chicago**
USDA	**United State Diabetes Association**
WHO	**World Health Organization**

ABSTRACT

Type 2 diabetes (T2D) is a progressive disorder with a consistent and steady increase in glycosylated hemoglobin (HbA1c) over time, associated with enhanced risk of micro- and macro vascular complications and a substantial reduction in life expectancy. It is a chronic disease associated with insulin resistance and a progressive failure of the pancreatic beta cells. The type 2diabetes is believed to account for about 90% of all cases of diabetes. Dyslipidemia is common feature in diabetic patients and considered to be responsible to a large extent for CVD-related morbidity and mortality. Since the micro vascular and macro vascular complications were reduced through strict glycemic and lipid control so this study was aimed to evaluate glycemic and lipid control of type 2 , patients It was conducted at Benghazi diabetic centre, for treatment and management of diabetes in Benghazi city. Our study was more focused on metformin (Glucophage) which is an oral antidiabetic drug belongs to the biguanide class. It is the first-line drug of choice for the treatment of type 2 diabetes, in particular, in overweight and obese people and those with normal kidney function. Metformin works by suppressing glucose production by the liver. Metformin is the only antidiabetic drug that has been conclusively shown to prevent the cardiovascular complications of diabetes. It reduces LDL-C ,cholesterol and triglycerides levels, and it does not associated with weight gain. A non-experimental, observational retrospective and prospective cohort was chosen as the means to investigate the efficacy and safety of metformin as a drug of the first choice in the treatment of newly diagnosed type 2 patients, A total of 60 newly diagnosed type 2 DM patients divided in to two groups according to the dose of metformin (1g& 2g/day) each group was selected for 12 weeks follow up, the comparisons were conducted between these two groups for body weight , FBG, PPBG, HbA1C, lipid profile and LFTs, RFTs and this parameters were recorded at the first visit and at the end of week 12.

The results showed that metformin caused modest weight reduction in the treated patients after 12 weeks of regular treatment and significant reduction in their BMI. This beneficial effect of metformin help on diabetes improvement though the decrease of insulin resistance and increase insulin receptors' sensitivity . The results also showed that

the patients in two groups shifted the target glycemic control with more significant control at dose of 2g of metformin. Our data also indicated that metformin has lipid improving effect at a dose of 2g/day and showed reduction on the levels of TC,TG, and LDL-C with low effect on HDL-C.

Our results indicated the safety profile of metformin throughout the normal values of LFTs & RFTs. The second part of study was retrospective analysis of medical records performed on patients with type 2 diabetes undergoing routine follow-up surveillance in diabetic center the FBG,PPBG, HbA1c, and lipid profile and LFTs, RFTs were evaluated . All plasmatic parameters were determined after a 12-hour overnight fast, except that of PPBG that were obtained two hours after lunch. The comparisons were conducted between three groups of diabetics amongst them 30 patients treated with metformin alone in a dose of 2g/day, patients undergoing combination of metformin (2g/day) & insulin (30-40 Unit/day), and the last group 30 patients undergoing combination therapy of metformin (2g/day),& insulin (20-40 Unit/day) & simvastatin (40mg/day). The results showed that all patients have good glycemic control , the highest significant decrease in HbA1c level was in the second group patients undergo combined therapy of insulin + metformin.

The results also indicated that no significant change in lipid profile of metformin treated group as compared to metformin + insulin treated group. The data also revealed a significant increase in the HDL-C level in metformin + insulin + simvastastin treated group as compared to group treated by metformin + insulin. Further more the result showed that Levels of TC, LDL-C were significantly decreased, in metformin + insulin + simvastatin treated group as compared to Metformin + insulin treated group with no change in TG level. As well as the Atherogenic index (AI) was significantly decreased, in patients treated by metformin + insulin + simvastatin. With regard to LFTs and RFTs of all patients were within the normal range except for bilirubin The data indicated that the level of bilirubin was significantly increased, in metformin +insulin + simvastatin treated group compared to the other treated type 2 diabetic patients. The result indicated that simvastatin produce significant reduction in TC, LDL-C levels at higher dose (40mg/ day) as compare to the lower tow doses (10 - 20mg/day).the result showed that simvastatin Produce a significant and dose dependent reduction in TC &LDL-C levels.

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CHAPTER- I

INTRODUCTION

I .1. BLOOD SUGAR

The blood sugar concentration or blood glucose level is the amount of glucose (sugar) present in the blood of a human or animal. The body naturally tightly regulates blood glucose levels as a part of metabolic homeostasis. Glucose is the primary source of energy for the body's cells, and blood lipids (in the form of fats and oils) are primarily a compact energy store. Glucose is transported from the intestines or liver to body cells via the bloodstream, and is made available for cell absorption via the hormone insulin, produced by the body primarily in the pancreas.
The mean normal blood glucose level in humans is about, (5.5 mmol/L or 100 mg/dL), however this level fluctuates throughout the day. Glucose levels are usually lowest in the morning, before the first meal of the day (termed "the fasting level"), and rise after meals for an hour or two by a few millimolar. The normal fasting blood glucose level for non-diabetics, should be between 70 and 100 mg/dL, and who are not fasting should be below 125 mg/dL. The blood glucose target range for diabetics, according to the American Diabetes Association(2006),should be (5 to 7.2 mmol/L or 70–130 mg/dL) before meals, and less than (10 mmol/L or180 mg/dL) after meals **(**Davidson et al. , 2011**).**

I.1.1. Unites of blood sugar measuring

The international standard way of measuring blood glucose levels are in terms of a molar concentration, measured in (mmol/L). In the United States, mass concentration is measured in (mg/dL). Since the molecular weight of glucose $C_6H_{12}O_6$ is about 180 g/mol, for the measurement of glucose, the difference between the two scales is a factor of 18, so that 1 mmol/L of glucose is equivalent to 18 mg/dL. (USDA.,2009)

I.1.2. Abnormality in blood glucose level

Glucose levels vary before and after meals, and at various times of day; the definition of "normal" varies among medical professionals. In general, the normal range for most people (fasting adults) is about 80 to 110 mg/dl or 4 to 6 mmol/l. (where 80 mg/dl is "optimal"). A subject with a consistent range above 126 mg/dl or 7 mmol/l is generally held to have hyperglycemia, where as consistent range below 70mg/dl or 4mmol/l is considered hypoglycemic. In fasting adults, blood plasma glucose should not exceed 126 mg/dL. Sustained higher levels of blood sugar cause damage to the blood vessels and to the organs they supply, leading to the complications of diabetes. Chronic hyperglycemia can be measured via the glycated hemoglobin (HbA1c) test. The definition of acute hyperglycemia varies from 8 to 15 **(**Giugliano et al .,1997).

I.1.2. 1. Hyperglycemia:

Hyperglycemia, or high blood sugar , is a condition in which an excessive amount of glucose circulates in the blood plasma. The origin of the term is Greek: *hyper-*, meaning excessive; *-glyc-*, meaning sweet; and *-emia*, meaning *of the blood.*
Hyperglycemia occurs when a glucose level exceeded 11.1mmol/l (200 mg/dl), but symptoms may not start to become noticeable until even higher values such as 15–20 mmol/l (~250–300 mg/dl). A subject with a consistent range between 100 and 126 according to ADA guidelines is considered hyperglycemic, while above 126 mg/dl or 7 mmol/l is generally held to have diabetes (Pais et al 2007**).**

I.1.2.2. Hypoglycemia:

Hypoglycemia is a medical emergency that involves an abnormally diminished content of glucose in the blood. It can produce a variety of symptoms and effects but the principal problems arise from an inadequate supply of glucose to the brain, resulting in impairment of function (neuroglycopenia). Effects can range from mild dysphoria to more serious issues such as seizures, unconsciousness, and (rarely) permanent brain damage or death. (*Stedman's Medical Dictionary*. 2005). The most common forms of hypoglycemia occur as a complication of treatment of diabetes mellitus with insulin or oral medications. Hypoglycemia is less common in non-diabetic persons, but can occur at any age. Among the causes are excessive insulin produced in the body, medications and poisons, alcohol, hormone deficiencies, prolonged starvation, alterations of metabolism associated with infection, and organ failure. Hypoglycemia is treated by restoring the blood glucose level to normal by the ingestion or administration of dextrose or carbohydrate foods. It is often self-diagnosed and self-medicated orally by the ingestion of balanced meals. In more severe circumstances it is treated by injection or infusion of glucagon. Recurrent hypoglycemia may be prevented by reversing or removing the underlying cause, by increasing frequency of meals, with medication likediazoxide,octreotide, or glucocorticoids, or removal of much of the pancreas. The level of blood glucose low enough to define hypoglycemia may be different for different people, in different circumstances, and for different purposes, and occasionally has been a matter of controversy. Most healthy adults maintain fasting glucose levels above 4.0 mmol/L (72 mg/dl), and develop symptoms of hypoglycemia when the glucose falls below 4 mmol/L (Cryer ., 2001).

I.2. Prediabetes:

A pre-diabetic state is a condition in which the fasting blood glucose is higher than the upper limit of normal, but not high enough to be classified as diabetes mellitus. As defined by WHO, people with prediabetes have impaired fasting glucose (IFG), with a fasting plasma glucose (FPG) concentration between 6·1 and 7·0 mmol/L. ADA uses a lower cutoff value for IFG (FPG 5·6–6·9 mmol/L).Some patients with IFG can also be diagnosed with impaired glucose tolerance (IGT),(but many have normal responses to a glucose tolerance test). associated with insulin resistance and increased risk of cardiovascular pathology, although of lesser risk than (IGT). IFG sometimes progresses to type 2 diabetes mellitus. There is a 50% risk over 10 years of progressing to overt diabetes. Many newly identified IFG patients progress to diabetes in less than three years (Nichols et al., 2007). IFG is also a risk factor for mortality **(**Barr et al., 2007**).**

WHO criteria: fasting plasma glucose level from 6.1 mmol/l (110 mg/dL) to 6.9 mmol/L (125 mg/dL).

ADA criteria: fasting plasma glucose level from 5.6 mmol/L (100 mg/dL) to 6.9 mmol/L (125 mg/dL).

I.3. Historical background of DM

Diabetes was one of the first diseases described with an Egyptian manuscript from c. 1500 BCE mentioning "too great emptying of the urine". The first described cases are believed to be of type 1 diabetes Indian physicians around the same time identified the disease and classified it as madhumeha or "honey urine", noting the urine would attract ants **(** Ripoll et al.,2011) .That diabetic urine tasted sweet was subsequently emphasized by Arabic Medical Texts during the 9^{th} – 11^{th} centuries, when Arabic medicine was at its peak of achievement. Avicenna (AD 960 - 1037) described accurately the clinical

feature of diabetes and mentioned two specific complications of the disease namely “gangrene” and “fall down” of sexual function. He recommended treatment of a mixture of lupin, fenugreek and zedoary seeds that possess mild hypoglycemic activity **(**Dobson.,197**7)**. The term "diabetes" or "to pass through" was first used in 230 BCE by the Greek Appollonius of Memphis.The disease was considered rare during the time of the Roman empire, with Galen commenting he had only seen two cases during his career **(** Leonid .,2009**)**. This is possibly due to the diet and life-style of the ancient people, or because the clinical symptoms were observed during the advanced stage of the disease. Galen named the disease "diarrhea of the urine" (diarrhea urinosa). The earliest surviving work with a detailed reference to diabetes is that of Aretaeus of Cappadocia (2nd or early 3rd century CE). He described the symptoms and the course of the disease, which he attributed to the moisture and coldness, reflecting the beliefs of the "Pneumatic School". He hypothesized a correlation of diabetes with other diseases and he discussed differential diagnosis from the snakebite which also provokes excessive thirst. His work remained unknown in the West until the middle of the 16th century. in 1552, the first Latin edition was published in Venice **(**Konstantinos et al.,2012**).** Type 1 and type 2 diabetes were identified as separate conditions for the first time by the Indian physicians Sushruta and Charaka in 400-500 CE with type 1 associated with youth and type 2 with being overweight. The term "mellitus" or "from honey" was added by the Briton John Rolle in the late 1700s to separate the condition from diabetes insipidus, which is also associated with frequent urination. Effective treatment was not developed until the early part of the 20th century, when Canadians Frederick Banting and Charles Herbert Best isolated and purified insulin in 1921 and 1922 This was followed by the development of the long-acting insulin NPH in the 1940s. (Leonid.,2009). The first oral hypoglycemic agent was discovered serendipitously in 1942 by M.J. Janbon, Professor of Pharmacology, while working on sulfonylurea for typhoid disease in Montpellier in France. He asked August Loubaieres, Professor of Medicine to try this agent on diabetic patients. Sulfonylurea produced an undoubted fall of blood glucose but it was ineffective in animals after pancreactomy. Ten years later, Franke and Fuchs in Berlin rediscovered the sulfonylurea as oral hypoglycemic agent and applied it clinically (Barnett and Krall.,2005).

I.4. Diabetes mellitus as global disease

Diabetes is one of the four priority non communicable diseases (NCDs) along with cardiovascular disease (CVD), cancer and chronic respiratory diseases. Once a disease of affluence, it is now increasingly common among the poor (Hu.,2011**).** Asia accounts for 60% of the world’s diabetic population.

DM affects 371 million people worldwide, and 187 million of them do not even know they have the disease, according to the International Diabetes Federation (IDF). Similar to other NCDs, the majority of people with diabetes (80%) live in low- and middle-income countries. While 4 million people died from the diabetes in 2011, estimates show that 4.8 million people will die this year from complications of the disease with people under 60 accounting for half the deaths. diabetes is the seventh leading cause of death in the U.S

The results were released on Nov. 14, World Diabetes Day 2012, in order to bring awareness to the global problem. Researchers estimate that the diabetes dilemma will only increase. By 2030, they expect 552 million people will have the disease representing around 10% of the global adult population if nothing else is done.

Type 1 DM represents (5 % of diagnosed cases), Type 2 diabetes represents (about 90 to 95% of diagnosed cases) and gestational diabetes represents (about 2 to 5% of diagnosed cases).

Already, $471 billion was spent treating the disease in 2012, up $6 billion from last year.

Four out of five people with diabetes live in low and middle class communities according to the IDF. North America spends the most to treat the disease. The CDC reports that 25.8 million people in the U.S. have diabetes, or about 8.3 percent of the population. About 7 million of them are diagnosed, and 79 million more people have the pre diabetes form of the disease.

Other highly-affected areas include the Western Pacific, where one out of three adults have the disease, as well as in South-East Asia where one out of four diabetes deaths take place. China has 92.3 million people who have diabetes, more than any other country (Michelle ., 2012**).**

In 2008, age-standardized adult diabetes prevalence was 10% in men and 9% in women **(**Danaei et al.,1980). Evidence shows that the burden of diabetes continues to shift to low and middle-income countries. Almost 80% of diabetes deaths occur in low- and middle-income countries and have limited access to affordable treatment **(**Roglic and Unwin.,2010).

The WHO predicted net losses in national income from diabetes and CVD of International Dollar (ID) 558 billion in China, ID 303 billion in the Russian Federation, and ID 237 billion in India between 2005 and 2015 **(**WHO.,2005**)** Health expenditure is astronomical in diabetes. In 2011 the disease caused at least US$ 465 billion dollars in healthcare expenditure.

This constitutes about 11% of the total health expenditure in adults**.** Diabetes kills and disables every eight seconds, somewhere in the world someone dies from diabetes A large proportion of the four million people who die each year as a result of diabetes are in their most productive years (40-60 years), resulting in a high economic cost to society. Almost half of diabetes deaths occur in people under the age of 70 years; 55% of diabetes deaths are in women.

The (IDF) estimated in 2011 that up to 50% of cases of gestational diabetes may end up as type 2 diabetes. The highest undiagnosed cases are in Africa. This is greater than the global mortality for hypertension, AIDS, and tuberculosis. The greatest number of people with diabetes is between 40 and 59 years of age **(** Oputa., 2012**).**

I. 5. Diabetes mellitus

The (WHO), defines diabetes as a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism that results from defects in insulin secretion, insulin action, or both. Diabetes is associated with reduced life expectancy; the significant morbidity associated with diabetes arises from micro vascular complications increased risk of macro vascular complications (ischemic heart disease, stroke, and peripheral vascular disease), and diminished quality of life (WHO.,1999).

I.5.1. Epidemiology of DM

Globally, as of 2010, an estimated 285 million people had diabetes, with type 2 making up about 90% of the cases **(**Shlomo et al .,2011). Its incidence is increasing rapidly, and by 2030, this number is estimated to almost double DM occurs throughout the world, but is more common (especially type 2) in the more developed countries. The greatest increase in prevalence is, however, expected to occur in Asia and Africa, where most patients will probably be found by 2030. The increase in incidence in developing countries follows the trend of urbanization and

lifestyle changes, perhaps most importantly a "Western-style" diet. This has suggested an environmental (i.e., dietary) effect (Wild et al., 2004**).** India has more diabetics than any other country in the world, according to the IDF, although more recent data suggest that China has even more. The disease affects more than 50 million Indians 7.1% of the nation's adults and kills about one million Indians a year. The average age on onset is 42.5 years(Gale and Jason., 2010**).**

I.5.2. Classification of DM

DM is classified into four broad categories: type1, type 2, gestational diabetes and "other specific types The "other specific types" are a collection of a few dozen individual causes (David et al., 2011). The term "type 1 diabetes" has replaced several former terms, including childhood-onset diabetes, juvenile diabetes, and insulin-dependent diabetes mellitus (IDDM). the term "type 2 diabetes" has replaced several former terms, including adult-onset diabetes, obesity-related diabetes, and noninsulin-dependent diabetes mellitus (NIDDM).

I.5.2.1.Type 1 diabetes

Type 1 diabetes mellitus is characterized by loss of the insulin-producing beta cells of the islets of Langerhans in the pancreas, leading to insulin deficiency. This type can be further classified as immune-mediated or idiopathic. The majority of type 1 diabetes is of the immune-mediated nature, in which beta cell loss is a T-cell-mediated autoimmune attack **(**Rother .,2007). There is no known preventive measure against type 1 diabetes, which causes approximately 10% of diabetes mellitus cases in North America and Europe. Most affected people are otherwise healthy and of a healthy weight when onset occurs. Sensitivity and responsiveness to insulin are usually normal, especially in the early stages. Type 1 diabetes can affect children or adults, but was traditionally termed "juvenile diabetes" because a majority of these diabetes cases were in children.

"Brittle" diabetes, also known as unstable diabetes or labile diabetes, is a term that was traditionally used to describe the dramatic and recurrent swings in glucose levels, often occurring for no apparent reason in insulin-dependent diabetes **(**Merck .,2010). There are many reasons for type 1 diabetes to be accompanied by irregular and unpredictable hyperglycemia, frequently with ketosis, and sometimes serious hypoglycemia, including an impaired counter regulatory response to hypoglycemia, occult infection, gastro paresis (which leads to erratic absorption of dietary carbohydrates), and endocrine pathies (e.g., Addison's disease), These phenomena are believed to occur no more frequently than in 1% to 2% of persons with type 1 diabetes **(**Dorner et al.,1977**).**

I.5.2.2.Type 2 diabetes

Type 2 diabetes mellitus is characterized by insulin resistance, which may be combined with relatively reduced insulin secretion. The defective responsiveness of body tissues to insulin is believed to involve the insulin receptor. Type 2 diabetes is the most common type (David and Dolores., 2011).

Feature	Type 1 diabetes	Type 2 diabetes
Onset	Sudden	Gradual
Age at onset	Mostly in children	Mostly in adults
Body habitus	Thin or normal	Often obese
Ketoacidosis	Common	Rare
Autoantibodies	Usually present	Absent
Endogenous insulin	Low or absent	Normal, decreased or increased
Concordance in identical twins	50%	90%
Prevalence	~10%	~90%

Table-1 Comparison of type 1 and 2 diabetes table (Shlomo et al.,2011.Williams 12th ed).

I.5.2.3.Gestational diabetes

Gestational diabetes mellitus (GDM) resembles type 2 diabetes in several respects, involving a combination of relatively inadequate insulin secretion and responsiveness. it occurs in about 2–5% of all pregnancies and may improve or disappear after delivery. Gestational diabetes is fully treatable, but requires careful medical supervision throughout the pregnancy. About 20–50% of affected women develop type 2 diabetes later in life. Though it may be transient, untreated gestational diabetes can damage the health of the fetus or mother.

Risks to the baby include

1- macrosomia (high birth weight),
2- congenital cardiac and central nervous system anomalies,
3- skeletal muscle malformations
4- respiratory distress syndrome (RDS)
Increased fetal insulin may inhibit fetal surfactant production and causes RDS.
5- Hyperbilirubinemia may result from red blood cell destruction. In severe cases, prenatal death may occur, most commonly as a result of poor placental perfusion due to vascular impairment
6- Labor induction may be indicated with decreased placental function.
7- Caesarean section may be performed if there is marked fetal distress or an increased risk of injury associated with macrosomia, such as shoulder dystocia.
A 2008 study completed in the U.S. found the number of American women entering pregnancy with pre-existing diabetes is increasing. In fact, the rate of diabetes in expectant mothers had more than doubled from 1999 to 2005. This is particularly problematic as diabetes raises the risk of complications during pregnancy and increases the potential for the children of diabetic mothers to become diabetic in the future (Lawrence et al., 2008).

I.5.2.4.Other types of diabetes

1- Prediabetes
It is a condition that occurs when a person's blood glucose levels are higher than normal but not high enough for a diagnosis of type 2 DM. Many people destined to develop type 2 DM spend many years in a state of prediabetes.

2-Latent autoimmune diabetes of adults
It is a condition in which type 1 DM develops in adults. Adults with LADA are frequently initially misdiagnosed as having type 2 DM, based on age rather than etiology. some cases of diabetes are caused by the body's tissue receptors not responding to insulin (even when insulin levels are normal, which is what separates it from type 2 diabetes); this form is very uncommon. genetic mutations (autosomal or mitochondrial) can lead to defects in beta cell function. Abnormal insulin action may also have been genetically determined in some cases.

3-Diabetes induced by diseases
Disease that causes extensive damage to the pancreas may lead to diabetes (for example, chronic pancreatitis and cystic fibrosis). Diseases associated with excessive secretion of insulin-antagonistic hormones can cause diabetes (which is typically resolved once the hormone excess is removed) (WHO.,1999).

I.5.3. Prevalence of DM

Diabetes mellitus occurs throughout the world, but is more common (especially type 2) in the more developed countries. The greatest increase in prevalence is, however, expected to occur in Asia and Africa. The increase in incidence in developing countries follows the trend of urbanization and lifestyle changes, perhaps most importantly a "Western-style" diet. This has suggested an environmental (i.e., dietary) effect, (Wild et al,2004**).**

Diabetes is an important and common health problem in Northern Africa. Variations in prevalence of diabetes between individual countries are observed. Chronic complications of diabetes are common. The highest increase is seen in urban areas changing patterns of diet, physical activity, and ageing populations are thought to be the major drivers of the increasing prevalence of diabetes in Africa. Cheap availability of high-fat and high-energy food in combination with less physical activity has led to the increasing prevalence of obesity .which can cause impaired glucose tolerance, which can lead to increased susceptibility to diabetes manifestation. The diabetes prevalence ranged from 2.6% in rural Sudan to 20.0% in urban Egypt. Prevalence was significantly higher in urban areas than in rural areas.

Undiagnosed diabetes is common in Northern Africa with a prevalence ranging from 18% to 75%. The prevalence of chronic diabetes complications ranged from 8.1% to 41.5% for retinopathy, 21% to 22% for albuminuria, 6.7% to 46.3% for nephropathy and 21.9% to 60% for neuropathy. Prevalence of retinopathy ranged from 8.1% in Tunisia to 41.5% in Egypt Albuminuria prevalence ranged from 21% in Egypt to 22% in Sudan (Manouk and Charles .,2013).and according to study done on 4000 Libyan individuals at the city of Tripoli, age between 18 and 65 years, 60% females. Early results showed that 73% of the individuals are diabetic or at high risk to have diabetes. About 70% of those individuals are obese (BMI >30%), and about 95% are obese and have family history of diabetes. Libya has highest prevalence in North Africa, the most possible cause is eating habit (Harrogate., 2009) .

I.5.3.1. prevalence of type 2 diabetes:

The prevalence of type 2 diabetes increased dramatically in the Arabic-speaking countries over the last three decades, a trend that parallels increased industrial development.

The wealth generated by oil-rich resources in countries of the Arabian Gulf have led to improved living standards, while there have also been accelerated urbanization, drastic changes in nutrition, reduced physical activity, and a greater reliance on mechanization.

As many as six Arabic-speaking countries are among the world's leaders in terms of type 2 diabetes prevalence: these countries are Kuwait, Lebanon, Qatar, Saudi Arabia, Bahrain, and United Arab Emirates (UAE) provides the 2010 IDF statistics for type 2 diabetes prevalence in developed and developing countries. An estimated 9.1% of the populations from the Middle Eastern/North African region have type 2 diabetes (32.8million) in 2011, and this is projected to reach 60 million in 2030. The explosion of type 2 diabetes in this region, within the 20–79 age groups, accounts for about 280,000 yearly deaths in the Middle Eastern/North African region, with mortality attributable to diabetes being equal in males (141,000) and females (138,000).

I.5.3.2. prevalence of type I diabetes

The Middle Eastern/North African region, Saudi Arabia has the largest ***number*** of cases (65,000) of type 1DM in children aged 0–14 years, while Kuwait has the highest incidence rate (Mohamed and Ismail., 2012).

I.5.4. Signs and symptoms of DM

1- unexplained weight loss
2- polyuria (frequent urination)
3- polydipsia (increased thirst)
4- polyphagia (increased hunger).
Symptoms may develop rapidly (weeks or months) in type 1 diabetes, while they usually develop much more slowly and may be subtle or absent in type 2 diabetes. Prolonged high blood glucose can cause glucose absorption in the lens of the eye, which leads to changes in its shape, resulting in vision changes. Blurred vision is a common complaint leading to a diabetes diagnosis. A number of skin rashes that can occur in diabetes are collectively known as diabetic dermatomes (Cooke and Plotnick.,2008)..

I.5.5. Risk factors of DM

1- Obesity
The number one risk factor for type 2 diabetes is obesity. The National Center for Health Statistics states that 30% of adults are obese and have a higher risk of insulin resistance, because fat interferes with the body's ability to use insulin. According to the same study, the number of overweight kids has tripled since 1980. The number of children being diagnosed with type 2 diabetes has also risen (Debra., 2010).

2- Sedentary Lifestyle and lack of regular exercise
A sedentary lifestyle is damaging to health and bears responsibility for the growing obesity problems. Muscle cells have more insulin receptors than fat cells, insulin resistance decreased by exercising.

3- Unhealthy Eating Habits –poor diet
About 90% of people who have been diagnosed with type 2 diabetes are overweight. Unhealthy eating contributes largely to obesity. Too much fat, not enough fiber, and too many simple carbohydrates all contribute to a diagnosis of diabetes.

4- Family History and Genetics
It appears that people who have family members who have been diagnosed with type 2 diabetes are at a greater risk for developing it themselves. African Americans, Hispanic-Americans and Native Americans all have a higher than normal rate of type 2 diabetes. Having a genetic disposition towards type 2 .

5- Increased Age
Scientists theorize that the pancreas ages right along with increase individual age, and become unable to pump insulin efficiently. Also, as the cells age, they become more resistant to insulin.

6- High Blood Pressure and High Cholesterol - dyslipidaemia
These two risk factors for many diseases and conditions, including type 2 diabetes. Because they are two key components in metabolic syndrome, so increases risk of heart disease, stroke, and diabetes**.**

7- History of Gestational Diabetes- and large babies

Gestational diabetes affects about 4% of all pregnant women. Placental hormones increase mother insulin resistant. Many women who have gestational diabetes develop type 2 diabetes years later. Their babies are also at some risk for developing diabetes later in life.

8- history of IGT and IFG, and Pre diabetes

People with fasting glucose levels from 110 to 125 mg/dl (6.1 to 6.9 mmol/l) are considered to have impaired fasting glucose**.** Patients with plasma glucose at or above 140 mg/dL (7.8 mmol/L), but not over 200 mg/dL (11.1 mmol/L), two hours after a 75 g oral glucose load are considered to have impaired glucose tolerance **(**Selvin et al., 2010).

I.5.6 Causes of DM

The cause of diabetes depends on the type :

Type 1 diabetes is partly inherited, and then triggered by certain infections, with some evidence pointing at Coxsackie B4 virus. However, even in those who have inherited the susceptibility, type 1 DM seems to require an environmental trigger. The onset of type 1 diabetes is unrelated to life style. Type 2 diabetes is due primarily to lifestyle factors and genetics (Riserus and willet .,2009).

The following is a comprehensive list of other causes of diabetes **(**Mitchell et al.,2009).

- **Genetic defects of β-cell function**
  - Maturity onset diabetes of the young
  - Mitochondrial DNA mutations
- **Genetic defects in insulin processing or insulin action**
  - Defects in proinsulin conversion
  - Insulin gene mutations
  - Insulin receptor mutations
- **Exocrine pancreatic defects**
  - Chronic pancreatitis
  - Pancreatectomy
  - Pancreatic neoplasia
  - Cystic fibrosis
  - Hemochromatosis
  - Fibrocalculous pancreatopathy
- **Endocrinopathies**
  - Growth hormone excess (acromegaly)
  - Cushing syndrome
  - Hyperthyroidism
  - Pheochromocytoma
  - Glucagonoma
- **Infections**
  - Cytomegalovirus infection
  - Coxsackievirus B
- **Drugs**
  - Glucocorticoids
  - Thyroid hormone
  - β-adrenergic agonists
  - Statins (Sattar et al., 2010).

1.5.7. Pathophysiology of DM

Normal glucose homeostasis is controlled by three interrelated processes. There is gluconeogenesis (glucose production that occurs in the liver), uptake and utilization of glucose by the peripheral tissues of the body and insulin secretion by the pancreatic islet cells. What triggers the production and release of insulin from the pancreas is the presence of glucose in the body. The main function of insulin is to increase the rate of transport of glucose into certain cells of the body such as striated muscles, fibroblasts, and fat cells. It is also necessary for transport of amino acids, glycogen formation in the liver and skeletal muscle, triglyceride formation from glucose, nucleic acid , and protein synthesis. Insulin enters cells by first binding to target insulin receptors. DM and some of those with prediabetes have impaired glucose tolerance in these individuals, blood glucose rises to abnormally high levels. This may be from a lack of pancreatic hormone release or failure of target tissues to respond to the insulin present or both (Cotran et al.,1999).

I.5.8. pathogenesis of DM :

I.5.8.1. Pathogenesis of type 1 DM

Type 1A DM results from autoimmune destruction of the insulin-producing beta cells in the islets of Langerhans. This process occurs in genetically susceptible subjects, that is probably triggered by one or more environmental agents, and usually progresses over many months or years during which the subject is asymptomatic and euglycemic. Thus, genetic markers for type 1A diabetes are present from birth. immune markers are detectable after the onset of the autoimmune process, and metabolic markers can be detected with sensitive tests once enough ß-cell damage has occurred, but before the onset of symptomatic hyperglycemia. This long latent period is a reflection of the large number of functioning beta cells that must be lost before hyperglycemia occurs. **Type1B** DM refers to non-autoimmune islet destruction (Type 1B diabetes). The pathogenesis of type 1A diabetes is quite different from that of type 2 diabetes mellitus, in which both decreased insulin release (not on an autoimmune basis) and insulin resistance play an important role. Genome-wide association studies indicate that type 1 and type 2 diabetes genetic loci do not overlap, although inflammation (eg: interleukin-1 mediated) may play a role in islet beta cell loss in both types (Massimo and Jean., 2013).

I.5.8.2. Pathogenesis of type 2 DM:

The pathogenesis of type 2 diabetes is complicated by several factors. Patients present with a combination of varying degrees of insulin resistance and relative insulin deficiency, and it is likely that both contribute to type 2 diabetes. Furthermore, each of the clinical features can arise through genetic or environmental influences, making it difficult to determine the exact cause in an individual patient. Moreover, hyperglycemia itself can impair pancreatic beta cell function and exacerbate insulin resistance, leading to a vicious cycle of hyperglycemia causing a worsening metabolic state.Type 2 diabetes is often accompanied by other conditions, including hypertension, high serum low-density-lipoprotein (LDL) cholesterol concentrations, and low serum high-density-lipoprotein (HDL) cholesterol concentrations that, like type 2 diabetes, increase cardiovascular risk. This constellation of clinical conditions is referred to as the metabolic syndrome. Hyperinsulinemia occurring in response to insulin resistance may play an important role in the genesis of these abnormalities. Increased free fatty acid levels, inflammatory cytokines from fat, and oxidative factors, have all been implicated in the pathogenesis of metabolic syndrome, type 2 diabetes, and their cardiovascular complications (David et al., 2012).

I.5.9. Diagnosis of DM

Diabetes mellitus is characterized by recurrent or persistent hyperglycemia, and is diagnosed by demonstrating any one of the following according to the WHO (2007).

- Fasting plasma glucose level ≥ 7.0 mmol/l (126 mg/dl)
- Plasma glucose ≥ 11.1 mmol/l (200 mg/dL) two hours after a 75 g oral glucose load as in a glucose tolerance test
- Symptoms of hyperglycemia and casual plasma glucose ≥ 11.1 mmol/l (200 mg/dl)
- Glycated hemoglobin (Hb A1C) ≥ 6.5% (Vijan .,2010).

A positive result, in the absence of unequivocal hyperglycemia, should be confirmed by a repeat of any of the above methods on a different days. It is preferable to measure a fasting glucose level because of the ease of measurement and the considerable time commitment of formal glucose tolerance testing, which takes two hours to complete and offers no prognostic advantage over the fasting test (Saydah et al., 2001). According to the current definition, two fasting glucose measurements above 126 mg/dl (7.0 mmol/l) is considered diagnostic for diabetes mellitus. People with fasting glucose levels from 110 to 125 mg/dl (6.1 to 6.9 mmol/l) are considered to have impaired fasting glucose**.** Patients with plasma glucose at or above 140 mg/dL (7.8 mmol/L), but not over 200 mg/dL (11.1 mmol/L), two hours after a 75 g oral glucose load are considered to have impaired glucose tolerance. Of these two prediabetic states, the latter in particular is a major risk factor for progression to full-blown diabetes mellitus, as well as cardiovascular disease (Santaguida et al ., 2008). Glycated hemoglobin is better than fasting glucose for determining risks of cardiovascular disease and death from any cause (Selvin et al.,2010).

I.5.9. 1. Criteria for the diagnosis of diabetes mellitus

1. Symptoms of DM plus casual plasma glucose concentration > 200 mg/dl (11.1 mmol/1).
Casual is defined as any time of day without regard to time since last meal. The classic symptoms of diabetes include polyuria, polydipsia, and unexplained weight loss.

2. FPG 2:126 mg/dl (7.0 mmol/1). Fasting is defined as no caloric intake for at least 8 h. or

3. PPBG >200 mg/dl (11.1 mmol/1) during an OGTT. The test should be performed as described in 2007 by WHO (2007).
using a glucose load containing the equivalent of 75-g anhydrous glucose dissolved in water.

The corresponding categories when the OGTT is used are the following:
• 2-hr post load glucose (2-h PG) < 140 mg/dl (7.8 mmol/1) = normal glucose
tolerance;
• 2-hr BG > 140 (7.8 mmol/1) and < 200 mg/dl (11.1 mmol/1) = IGT
• 2-hrBG > 200 mg/dl (11.1 mmol/1)
provisional diagnosis of diabetes (the diagnosis must be confirmed, as described above). Since the 2-h OGTT cutoff of 140 mg/dl (7.8 mmol/1) will identify more people as having impaired glucose homeostasis than will the fasting cutoff of 110 mg/dl (6.1 mmol/1)

I.5.9.2 Criteria for testing for diabetes in asymptomatic, undiagnosed individuals

1. Testing for diabetes should be considered in all individuals at age 45 years and above and, if normal, it should be repeated at 3-year intervals.
2. Testing should be considered at a younger age or be carried out more frequently in individuals who:
• are obese (2:120% desirable body weight or a BMI >27 kg/m2)
• have a first-degree relative with diabetes (James .,1998).

Condition	*2 hour glucose Mmol/l(mg/d)l*	*Fasting glucose mmol/l(mg/dl)*	*HbA_{1c} %*
Normal	*<7.8 (<140)*	*<6.1 (<110)*	*<6.0*
Impaired fasting glycaemia	*<7.8 (<140)*	*≥ 6.1(≥110) & <7.0(<126)*	*6.0–6.4*
Impaired glucose tolerance	*≥7.8 (≥140)*	*<7.0 (<126)*	*6.0–6.4*
Diabetes mellitus	*≥11.1 (≥200)*	*≥7.0 (≥126)*	*≥6.5*

Table - 2 Diabetes diagnostic criteria (Vijan.,201n.,2010)

I.5.9.3. HbA1c as diagnostic and screening test for DM

Glycated hemoglobin or glycosylated hemoglobin (hemoglobin A1c, HbA_{1c}, A1C, or Hb_{1c}; sometimes also HbA1c) is a form of hemoglobin that is measured primarily to identify the average plasma glucose concentration over prolonged periods of time. It is formed in a non-enzymatic glycation pathway by hemoglobin's exposure to plasma glucose. Normal levels of glucose produce a normal amount of glycated hemoglobin. As the average amount of plasma glucose increases, the fraction of glycated hemoglobin increases in a predictable way. This serves as a marker for average blood glucose levels over the previous months prior to the measurement.In diabetes mellitus, higher amounts of glycated hemoglobin, indicating poorer control of blood glucose levels, have been associated with cardiovascular disease, nephropathy, and retinopathy. Monitoring HbA_{1c} in type 1 diabetic patients may improve outcomes (Larsen et al., 1990).

I.5.9.3.a. History of HbA1c

HbA1c was first separated from other forms of hemoglobin by Huisman and Meyering in 1958 using a chromatographic column (Huisman et al .,1958). It was first characterized as a glycoprotein by Bookchin and Gallop in 1968 (Bookchin and Gallop.,1968). Its increase in diabetes was first described in 1969 by Samuel Rahbar (Rahbar et al .,1969). The reactions leading to its formation were characterized by Bunn and his coworkers in 1975 (Bunn et al .,1975). The use of hemoglobin A1c for monitoring the degree of control of glucose metabolism in diabetic patients was proposed in 1976 by Anthony Cerami, Ronald Koenig and coworkers (Koenig et al .,1976).

I.5.9.3.b Principle

Glycation of proteins is a frequent occurrence, but in the case of hemoglobin, a nonenzymatic reaction occurs between glucose and the N-end of the beta chain. This forms a Schiff base which is itself converted to 1-deoxyfructose. This rearrangement is known as Amadori rearrangement.

When blood glucose levels are high, glucose molecules attach to the hemoglobin in red blood cells. The longer hyperglycemia occurs in blood, the more glucose binds to hemoglobin in the red blood cells and the higher the glycated hemoglobin.

Glucose levels are intermittently raised in portal vessels carrying absorbed glucose to the liver for regulation. Passing red cells will have increased glycation after sugary drink or porridge

Once a hemoglobin molecule is glycated, it remains that way. A buildup of glycated hemoglobin within the red cell, therefore, reflects the average level of glucose to which the cell has been exposed during its life-cycle. Measuring glycated hemoglobin assesses the effectiveness of therapy by monitoring long-term serum glucose regulation. The HbA_{1c} level is proportional to average blood glucose concentration over the previous four weeks to three months. Some researchers state that the major proportion of its value is weighted toward the most recent 2 to 4 weeks (Bunn.,1975).

I.5.9.3.c. Measuring of HbA1C

Hb A1C measures by High-performance liquid chromatography (HPLC): The HbA1c result is calculated as a ratio to total hemoglobin by using a chromatogram, Immunoassay, Enzymatic,Capillary electrophoresis, Boronate affinity chromatography. Results can be unreliable in many circumstances, such as after blood loss, for example, after surgery, blood transfusions, anemia, or high erythrocyte turnover; in the presence of chronic renal or liver disease; after administration of high-dose vitamin C; or erythropoetin treatment. In general, the reference range (that found in healthy persons), is about 20–40 mmol/mol (4–5.9%) (Nathan et al., 2008). Higher levels of HbA_{1c} are found in people with persistently elevated blood sugar, as in diabetes mellitus. While diabetic patient treatment goals vary, many include a target range of HbA_{1c} values. A diabetic person with good glucose control has a HbA_{1c} level that is close to or within the reference range. American College of Endocrinology recommend HbA_{1c} values below 48 mmol/mol (6.5%), while ADA recommends that the HbA_{1c} be below 53 mmol/mol (7.0%) for most patients. Recent results from large trials suggest that a target below 53 mmol/mol (7%) may be excessive: Below 53 mmol/mol (7%) the health benefits of reduced HbA1C become smaller, and the intensive glycemic control required to reach this level leads to an increased rate of dangerous hypoglycemic episodes (Lehman and Krumholz., 2009).

The risks of the main complications of diabetes (retinopathy, nephropathy, neuropathy and macrovascular disease) decreased with approximately 3% for every 1 mmol/mol decrease in HbA_{1c} (Shubrook ., 2010). Persistent elevations in blood sugar (and, therefore, HbA_{1c}) increase the risk of long-term vascular complications of diabetes such as coronary disease, heart attack, stroke, heart failure, kidney failure, blindness, erectile dysfunction, neuropathy (loss of sensation, especially in the feet), gangrene, and gastroparesis (slowed emptying of the stomach). Poor blood glucose control also increases the risk of short-term complications of surgery such as poor wound healing. Lower-than-expected levels of HbA_{1c} can be seen in people with shortened red blood cell lifespan, such as with glucose-6-phosphate dehydrogenase deficiency, sickle-cell disease, or any other condition causing premature red blood cell death. Blood donation will result in rapid replacement of lost RBCs with newly formed red blood cells. Since these new RBCs will have only existed for a short period of time, their presence will lead HbA1c to underestimate the actual average levels. There may also be distortions resulting from blood donation which occurred as long as two months before due to an abnormal synchronization of the age of the RBCs, resulting in an older than normal average blood cell life (resulting in an overestimate of actual average blood glucose levels). Conversely, higher-than-expected levels

can be seen in people with a longer red blood cell lifespan, such as with Vitamin B_{12} or folate deficiency (Kilpatrick et al ., 2009).

I.5.9.3.d. Indications and use of HbA1c

Glycated hemoglobin testing is recommended for both
(a) checking the blood sugar control in people who might be pre-diabetic and
(b) monitoring blood sugar control in diabetic patients. There is a significant proportion of people who are unaware of their elevated HbA_{1c} level before they have blood lab work (Walid et al., 2009). For a single blood sample, it provides far more revealing information on glycemic behavior than a fasting blood sugar value. However, fasting blood sugar tests are crucial in making treatment decisions. The ADA guidelines are similar to others in advising that the HbA1c test be performed at least two times a year in patients with diabetes that are meeting treatment goals (and that have stable glycemic control) and quarterly in patients with diabetes whose therapy has changed or that are not meeting glycemic goals (ADA., 2007).

I.9.3.e. Use of HbA1c in the Diagnosis of DM

HbA1c was introduced into clinical use in the 1980s and subsequently has become a cornerstone of clinical practice (Massi., 2006).
It can be performed at any time of the day and does not require any special preparation such as fasting. It is used as diagnostic test for diabetes and as a screening test for persons at high risk of diabetes . HbA1c has now been recommended by an International Committee and by the ADA as a means to diagnose diabetes. Although it gives equal or almost equal sensitivity and specificity to a fasting or post-load glucose measurement as a predictor of prevalent retinopathy. The use of HbA1c can avoid the problem of day-to-day variability of glucose values, and importantly it avoids the need for the person to fast and to have preceding dietary preparations. These advantages have implications for early identification and treatment which have been strongly advocated in recent years.
A recent report from Australia has shown that a model including HbA1c for predicting incident retinopathy is as good as or possibly better than one including fasting plasma glucose (Tappr et al., 2008).
A report published in 2009 by an International Expert Committee (IEC) on the role of HbA1c in the diagnosis of diabetes recommended that HbA1c can be used to diagnose diabetes and that the diagnosis can be made if the HbA1c level is 6.5%. Diagnosis should be confirmed with a repeat HbA1c test, unless clinical symptoms and plasma glucose levels >11.1mmol/l (200 mg/dl) are present in which case further testing is not required. Levels of HbA1c just below 6.5% may indicate the presence of intermediate hyperglycemia. The ADA (2006) has suggested
5.7 – 6.4% as the high risk range. While recognizing the continuum of risk that may be captured by the HbA1c assay, the IEC recommended that persons with a HbA1c level between 6.0 and 6.5% were at particularly high risk and might be considered for diabetes prevention interventions.

I.5.9.3. f. Modification of HbA1c by exercise training

A meta-analysis of research done to identify the effect of two different kinds of training programs (combined aerobic and eccentric resistance exercise program and aerobic exercise only) on the HbA1c levels of individuals with Type 2DM found that the effect of combining resistance exercise with aerobic exercise improved the glucose control more than just the aerobics alone. The average effect of the training programs included reductions of HbA1C of 9 mmol/mol (0.8 percentage points), which was a result similar to that of long-term diet and drug or insulin therapy (Marcus et al ., 2008).

I.5.9.3.g. Factors that influence HbA1c and its measurement

(**1**). Acute and chronic blood loss:
This decreases the red cell survival rate, so a decrease in the HbA1c levels.

(**2**). Haemolytic anaemias:
Similarly, due to decreased red cell survival, the Hb A1c is lowered.

(**3**). Blood urea:
In patients with uraemia and normal glucose tolerance, glycated haemoglobin measured by ion exchange chromatography was significantly elevated, This is due to the excessive amount of cyanate derived from the urea, which causes increase in carbamylated aemoglobin results in the increased levels of HbA1c.

(**4**). In chronic renal failure:
Patients on haemolysis and sometimes the gastrointestinal loss of blood lowers the HbA1c levels.

(**5**). Pregnancy:
Studies have shown that HbA1c levels decrease during the second trimester of a normal nondiabetic pregnancy and rise during the third trimester.

(**6**). Other anaemias:
The HbA1 values were significantly higher in iron & B12 deficiency anemic patients and the level decreased after treatment with iron & B12. The mechanism leading to increased HbA1 levels was not clear.

(**7**). Erythropoiesis:
Decreased erythropoiesis increase HbA1c level then Decreased after administration of erythropoietin.

(**8**) Altered Haemoglobin:
Genetic or chemical alterations in haemoglobin like haemoglobinopathies, methaemoglobin, may increase or decrease HbA1c.

(**9)**Disease:
Increased HbA1c in hyperbilirubinaemia, spleenoectomy (increases erythrocyte life span)
Decreased HbA1c in hypertriglyceridaemia.and splenomegaly, rheumatoid arthritis or drugs such as antiretrovirals ribavirin and dapsone (Gallagher et al., 2009 and Ninin., 2010).

I.5.10. Complications of DM

They are far less common and less severe in people who have well-controlled blood sugar levels (Nathan et al.,2005).Wider health problems accelerate the deleterious effects of diabetes. These include smoking, obesity, high blood pressure, elevated cholesterol levels and lack of regular exercise**.** (Gallagher et al.,2009).

I.5.10. 1. Acute complication of DM

I.5.10.1.a. Diabetic ketoacidosis

Diabetic ketoacidosis (DKA) is a potentially life-threatening complication in patients with diabetes mellitus. It happens predominantly in those with type 1 diabetes, but it can occur in those with type 2 diabetes under certain circumstances. DKA results from a shortage of insulin; in response the body switches to burning fatty acids and producing acidic ketone bodies that cause most of the symptoms and complications (Kitabchi et al., 2009).

DKA may be the first symptom of previously undiagnosed diabetes, but it may also occur in people known to have diabetes as a result of a variety of causes. DKA is a medical emergency, and without treatment it can lead to death. DKA was first described in 1886; until the introduction of insulin therapy in the 1920s it was almost universally fatal.(Eledrisi et al ., 2006). It now carries a mortality of less than 1% with adequate and timely treatment. The signs and symptoms of an episode of diabetic ketoacidosis usually evolve over the period of about 24 hours. Predominant symptoms are nausea and vomiting, pronounced thirst, excessive urine production and abdominal pain that may be severe. Those who measure their glucose levels themselves may notice hyperglycemia (high blood sugar levels). In severe DKA, breathing becomes labored and of a deep, gasping character (a state referred to as "Kussmaul respiration") (Kasper et al ., 2005). The abdomen may be tender to the point that an acute abdomen may be suspected, such as acute pancreatitis, appendicitis or gastrointestinal perforation. Coffee ground vomiting (vomiting of altered blood) occurs in a minority of patients; this tends to originate from erosion of the esophagus (Eledrisi et al ., 2006). In severe DKA, there may be confusion, lethargy, stupor or even coma (a marked decrease in the level of consciousness).

I.5.10.1.a.i . Mechanism of DKA

Diabetic ketoacidosis arises because of a lack of insulin in the body. The lack of insulin and corresponding elevation of glucagon leads to increased release of glucose by the liver (a process that is normally suppressed by insulin) from glycogen via glycogenolysis and also through gluconeogenesis. High glucose levels spill over into the urine, taking water and solutes (such as sodium and potassium) along with it in a process known as osmotic diuresis. This leads to polyuria, dehydration, and compensatory thirst and polydipsia. The absence of insulin also leads to the release of free fatty acids from adipose tissue (lipolysis), which are converted, again in the liver, into ketone bodies (acetoacetate and β-hydroxybutyrate). β-Hydroxybutyrate can serve as an energy source in the absence of insulin-mediated glucose delivery, and is a protective mechanism in case of starvation. The ketone bodies, however, have a low pKa and therefore turn the blood acidic (metabolic acidosis). The body initially buffers the change with the bicarbonate buffering system, but this system is quickly overwhelmed and (Kitabch et al., 2006). One such mechanism is hyperventilation to lower the blood carbon dioxide levels (a form of compensatory respiratory alkalosis). This hyperventilation, in its extreme form, may be observed as Kussmaul respiration (Kasper et al., 2005).

I.5.10.1.a. ii. Diagnosis of DKA

Diabetic ketoacidosis is distinguished from other diabetic emergencies by the presence of large amounts of ketones in blood and urine, and marked metabolic acidosis. Hyperosmolar

hyperglycemic state (HHS, sometimes labeled "hyperosmolar non-ketotic state" or HONK) is much more common in type 2 diabetes and features increased plasma osmolarity (above 320 mosm/kg) due to profound dehydration and concentration of the blood; mild acidosis and ketonemia may occur in this state, but not to the extent observed in DKA. There is a degree of overlap between DKA and HHS, as in DKA the osmolarity may also be increased.

Ketoacidosis is not always the result of diabetes. It may also result from alcohol excess and from starvation; in both states the glucose level is normal or low. Metabolic acidosis may occur in people with diabetes for other reasons, such as poisoning with ethylene glycol or paraldehyde.

The ADA categorizes DKA in adults into one of three stages of severity (Kitabch et al., 2006).

- *Mild:* blood pH mildly decreased to between 7.25 and 7.30 (normal 7.35–7.45); serum bicarbonate decreased to 15–18 mmol/l (normal above 20); the patient is alert
- *Moderate:* pH 7.00–7.25, bicarbonate 10–15, mild drowsiness may be present
- Severe: pH below 7.00, bicarbonate below 10, stupor or coma may occur

I.5.10.1.a.iii Mangment of DKA

- **Fluid replacement**

The amount of fluid depends on the estimated degree of dehydration. If dehydration is so severe as to cause shock (severely decreased blood pressure with insufficient blood supply to the body's organs), or a depressed level of consciousness, rapid infusion of saline . Very mild ketoacidosis with no associated vomiting and mild dehydration may be treated with oral rehydration and subcutaneous rather than intravenous insulin under observation for signs of deterioration (Edge., 2009).

- **Insulin**

Some guidelines recommend a bolus (initial large dose) of insulin of 0.1 unit of insulin per kilogram of body weight (Kitabch et al., 2006).

- **Potassium**

Potassium levels can fluctuate severely during the treatment of DKA, because insulin decreases potassium levels in the blood by redistributing it into cells. A large part of the shifted extracellular potassium would have been lost in urine because of osmotic diuresis. Hypokalemia . increases the risk of dangerous irregularities in the heart rate. Therefore, continuous observation of the heart rate is recommended.

Potassium should be added to the intravenous fluids once levels fall below 5.3 mmol/l (Edge., 2009).

I.5.10.1.b. Cerebral edema

Cerebral edema, if associated with coma, often necessitates admission to intensive care, artificial ventilation, and close observation. The administration of fluids is slowed. The ideal treatment of cerebral edema in DKA is not established, but intravenous mannitol and hypertonic saline (3%) are used as in some other forms of cerebral edema in an attempt to reduce the swelling (Dunger et al., 2004).

I.5.10.1.c . Hyperosmolar hyperglycemic state

(HHS) is a complication of diabetes mellitus (predominantly type 2) in which high blood sugars cause severe dehydration, increases in osmolarity and a high risk of complications, coma and death. It is diagnosed with blood tests. It is related to DKA , another complication of diabetes more often (but not exclusively) encountered in people with type 1 diabetes; they are differentiated with measurement of ketone bodies, organic molecules that are the underlying driver for DKA but are usually not detectable in HHS.

The treatment of HHS consists of correction of the dehydration with intravenous fluids, reduction of the blood sugar levels with insulin, and management of any underlying conditions that might have precipitated the illness, such as an acute infection.

I.5.10.1.d Hypoglycemia

It is an acute complication of several diabetes treatment. amount of glucose in the blood falls, the brain is one of the first organs affected. In most people, subtle reduction of mental efficiency can be observed when the glucose falls below 65 mg/dl (3.6 mM). Impairment of action and judgment usually becomes obvious below 40 mg/dl (2.2 mM). Seizures may occur as the glucose falls further. As blood glucose levels fall below 10 mg/dl (0.55 mM), most neurons become electrically silent and nonfunctional, resulting in coma. These brain effects are collectively referred to as neuroglycopenia. Prolonged severe hypoglycemia can produce permanent brain damage. It has been frequently found that those Type 1 diabetics found "dead in bed" in the morning after suspected severe hypoglycemia. It is treated by restoring the blood glucose level to normal by the ingestion or administration of dextrose or carbohydrate foods. It is often self-diagnosed and self-medicated orally by the ingestion of balanced meals. In more severe circumstances, it is treated by injection or infusion of glucagon. Recurrent hypoglycemia may be prevented by reversing or removing the underlying cause, by increasing the frequency of meals, with medications like diazoxide, octreotide, or glucocorticoids, or by surgical removal of much of the pancreas (Allen et al ., 1992).

I.5.10.1.e. Diabetic coma

Diabetic coma is a medical emergency in which a person with diabetes mellitus is comatose because of one of the acute complications of diabetes:

(**1**).Severe diabetic hypoglycemia
(**2**).DKA advanced enough to result in unconsciousness from a combination of severe hyperglycemia, dehydration and shock, and exhaustion
(**3**).HONK in which extreme hyperglycemia and dehydration alone are sufficient to cause unconsciousness (Dunger et al., 2004)

I.5.10.1. f. Respiratory infections

The immune response is impaired in individuals with diabetes mellitus. Cellular studies have shown that hyperglycemia both reduces the function of immune cells and increases inflammation. The vascular effects of diabetes also tend to alter lung function, all of which leads to an increase in susceptibility to respiratory infections such as pneumonia and influenza among individuals with diabetes. Several studies also show diabetes

associated with a worse disease course and slower recovery from respiratory infections (Ahmed et al., 2008).

I.5.10.1.g. Periodontal disease

Diabetes is associated with periodontal disease (gum disease) and may make diabetes more difficult to treat Gum disease is frequently related to bacterial infection (Mombelli ., 2012).

I.5.10.2. Chronic complications of DM

Microvascular disease" (due to damage to small blood vessels)
Macrovascular disease" (due to damage to the arteries).

I.5.10.2.a. Mechanisms of chronic complications

Chronic elevation of blood glucose level leads to damage of blood vessels (angiopathy). The endothelial cells lining the blood vessels taking more glucose than normal, since they do not depend on insulin. They then form more surface glycoproteins than normal, and cause the basement membrane to grow thicker and weaker. In diabetes, the resulting problems are grouped under "microvascular disease" (due to damage to small blood vessels) and "macrovascular disease" (due to damage to the arteries).

Recent research suggests that in type 1 diabetics, the continuing autoimmune disease which initially destroyed the beta cells of the pancreas may also cause retinopathy, neuropathy, and nephropathy (Grnberg et al., 2005)**.** One researcher has even suggested that retinopathy may be better treated by drugs to suppress the abnormal immune system of diabetics than by blood sugar control (Rich., 2006).

I.5.10.2.b. classification of chronic complication of DM

I .5.10.2.b.i. Microvascular disease

The damage to small blood vessels leads to a microangiopathy, which can cause one or more of the following :

- Diabetic cardiomyopathy, damage to the heart, leading to diastolic dysfunction and eventually heart failure.
- Diabetic nephropathy, damage to the kidney which can lead to chronic renal failure, eventually requiring dialysis. Diabetes mellitus is the most common cause of adult kidney failure worldwide in the developed world.
- Diabetic neuropathy, abnormal and decreased sensation, usually in a 'glove and stocking' distribution starting with the feet but potentially in other nerves, later often fingers and hands. When combined with damaged blood vessels this can lead to diabetic foot . Other forms of diabetic neuropathy may present as mononeuritis or autonomic neuropathy. Diabetic amyotrophy is muscle weakness due to neuropathy.
- Diabetic retinopathy, growth of friable and poor-quality new blood vessels in the retina as well as macular edema , which can lead to severe vision loss or blindness. Retinal damage (from microangiopathy) makes it the most common cause of blindness among non-elderly adults in the US.

I.5.10.2.b.ii. **Macrovascular disease**

leads to cardiovascular disease, to which accelerated atherosclerosis is a contributor:

- Coronary artery disease, leading to angina or myocardial infarction ("heart attack")
- Diabetic myonecrosis ('muscle wasting')
- Peripheral vascular disease, which contributes to intermittent claudication (exertion-related leg and foot pain) as well as diabetic foot.
- Stroke (mainly the ischemic type)

Diabetic foot, often due to a combination of sensory neuropathy (numbness or insensitivity) and vascular damage, increases rates of skin ulcers (diabetic foot ulcers) and infection and, in serious cases, necrosis and gangrene. It is why diabetics are prone to leg and foot infections and why it takes longer for them to heal from leg and foot wounds. It is the most common cause of non-traumatic adult amputation, usually of toes and or feet, in the developed world (Scott., 2013).

Carotid artery stenosis does not occur more often in diabetes, and there appears to be a lower prevalence of abdominal aortic aneurysm. However, diabetes does cause higher morbidity, mortality and operative risks with these conditions (Weiss and Sumpio.,2006).

Diabetic encephalopathy (Aristides ,et al .,2007). is the increased cognitive decline and risk of dementia- including (but not limited to) the Alzheimer's type- observed in diabetes. Various mechanisms are proposed, including alterations to the vascular supply of the brain and the interaction of insulin with the brain itself (Gispen and Biessels ., 2000).

In the developed world, diabetes is the most significant cause of adult blindness in the non-elderly and the leading cause of non-traumatic amputation in adults, and diabetic nephropathy is the main illness requiring renal dialysis in the United States (Mailloux and Lionel., 2007).

A review of type 1 diabetes showed that women with diabetes are at increased risk of female infertility, such as reflected by delayed puberty and menarche, menstrual irregularities (especially oligomenorrhoea), mild hyperandrogenism, polycystic ovarian syndrome, fewer live born children and possibly earlier menopause (Codner et al ., 2012).

I.5.11. Management of DM

Diabetes mellitus is a chronic disease, for which there is no known cure except in very specific situations. Management concentrates on keeping blood sugar levels as close to normal ("euglycemia") as possible, without causing hypoglycemia. This can usually be accomplished with diet, exercise, and use of appropriate medications (insulin in the case of type 1 diabetes; oral medications, as well as possibly insulin, in type 2 diabetes). The treatment goals for type 2 diabetic patients are related to effective control of blood glucose, blood pressure and lipids, to minimize the risk of long-term consequences associated with diabetes. They are suggested in clinical practice guidelines released by various national and international diabetes agencies.
The targets are:

- Hb_{A1c} of 6% to 7.0%
- FBG: 4.0 to 6.0 mmol/L (72 to 108 mg/dl)
- PPBG: 5.0 to 8.0 mmol/L(90 to 144 mg/dl) (Qaseem et al.,2007).
- . Hypertension Goals for DM
- Blood pressure*: < 140/80* (<130/80 for younger patients)
  Treatment options for hypertension:

-Sodium restriction
-ACE inhibitor/ARB
-Smoking cessation
-Monitor Blood pressure every 3 months (or every 1-2 weeks if not at goal)

- Hyperlipidemia Goals for DM
  Total cholesterol: < 200
  LDL: < 100
  HDL: > 50
  TGs: < 150

Treatment: lifestyle modifications, Statin therapy is gold standard for pharmacological therapy
Focus on LDL control unless TGs > 500
Aspirin Therapy (75-162 mg daily) is recommended for primary prevention in Type 1 or Type 2 diabetics at increased cardiovascular risk (10-year risk >10%) (Michelle.,2012).

General objectives of diabetes management

• To relieve symptoms

• To correct associated health problems and to reduce morbidity, mortality and economic costs of diabetes

• To prevent as much as possible acute and long-term complications; to monitor the development of such complications and to provide timely intervention

• To improve the quality of life and productivity of the individual with diabetes.

I.5.11.1. Management of long term complication of DM

I.5.11.1.a. Retinopathy

Diabetic retinopathy is a leading cause of visual disability. Significant retinopathy is rarely encountered in the first five years of insulin-dependent diabetes mellitus, nor before up to 20% may be found to have retinopathy at the time of first diagnosis of diabetes and most develop some degree of retinopathy over subsequent decades. Hypertension is an established risk factor .
Good control of diabetes results in reduction in the occurrence of retinopathy. Timely laser photocoagulation has been demonstrated to prevent a major proportion of severe visual loss associated with proliferative retinopathy.
Assessment should be performed every one-to-two years. If retinopathy is detected, follow-up should be arranged in one year or more frequently, if required.
To prevent retinopathy and visual loss, the following are recommended:
• promoting good glycaemic control in all diabetic individuals
• controlling blood pressure
• detecting and treating glaucoma at an early stage
• detecting and treating cataract
• detecting and providing timely treatment of potentially serious retinal changes (Alwan ., 1994**).**

I.5.11.1.b Nephropathy

Diabetic nephropathy is a major cause of death among people with diabetes and an important cause of morbidity and increased health care costs due to diabetes. It leads to end-stage renal disease requiring dialysis or renal transplantation.
This complication may be prevented and progression can be slowed by:
• strict glycaemic control
• vigorous treatment of hypertension

• avoidance of nephrotoxic drugs and early and effective treatment of infection.
The onset of clinical nephropathy is manifested by proteinuria. However, an earlier marker of the onset of nephropathy is the presence of microalbuminuria (defined as an overnight excretion of 20-200 microg/min or excretion of >30 mg/24-hr) on more than one occasion (Alwan .,1994**).**

I.5.11.1.c. Neuropathy

Neuropathy is a common complication of diabetes. It causes clinical manifestations and disabilities of diverse spectrum and considerable severity. Both peripheral nerves (sensory and motor) and the autonomic nervous system can be affected. Patients present with distal symmetrical polyneuropathy, focal neuropathy or manifestations of autonomic involvement such as gastroparesis, constipation, diabetic diarrhoea, bladder dysfunction, impotence and orthostatic hypotension.
Peripheral nerve affection together with peripheral vascular disease predispose to foot ulcers and infection. If not detected early, these lesions may progress to gangrene and result in amputation.
Neuropathic involvement can be prevented or delayed by good glycaemic control. Foot complications can be avoided by good foot care and detection of early lesions , Pain due to neuropathy can be severe and distressing and often requires attention.
If it persists in spite of good blood glucose control, drug treatment may be indicated.
Analgesics may be given but if ineffective, tricyclic antidepressants such as amitriptyline may also be used for this purpose. Reassurance that pain will eventually decrease with time is needed.
Diabetic gastropathy, caused by autonomic involvement, is often manifested by troublesome gastrointestinal symptoms such as heartburn, nausea and vomiting.
Symptoms may be relieved by agents promoting gastric emptying such as metoclopramide or domperidone (Alwan ., 1994**).**

I.5.11.1.d. Foot care

Severe foot lesions requiring amputation are one of the major complications of diabetes.
The two main approaches to prevention are:
(1) identification of high-risk individuals.
(2) early detection of foot lesions: for example, trauma, infection or ulcers.
Intensified foot care should be ensured for patients at high risk, such as those with :

- symptoms and/or signs of neuropathic involvement
- evidence of peripheral vascular disease
- nephropathy or significant retinopathy
- foot deformities and chronic orthopaedic or rheumatic disorders, and
- poor hygiene.

Instructions on foot care should be an integral part of any educational activity on diabetes diabetes.
They should focus on:

- Self-examination
- avoidance of trauma
- cessation of smoking, and
- wearing properly fitting shoes.

Efforts should be intensified in respect of high-risk people. Health-care professionals ,other than doctors, at the primary health care level should be trained to identify such individuals and recognize early lesions. Patients with suspected or confirmed abnormalities should be sent for medical consultation (Alwan ., 1994**).**

I.5.11.1.e. Cardiovascular diseases

Cardiovascular diseases (coronary heart disease and strokes) are the leading causes of death in the diabetic population. Risk factors for the development of macrovascular disease are frequently found in people with diabetes.

The initial assessment of the newly diagnosed type 2 DM individual should always include:

- clinical screening for risk factors of cardiovascular diseases (CVD); for example hypertension, smoking, obesity, and hyperlipidemia
- screening for early signs of cardiovascular abnormalities
- a baseline electrocardiogram
- serum lipid measurement, whenever possible.

Activities to reduce CVD risk factors should be an integral part of the management plan.

The management plan should include:

- prevention and cessation of smoking
- correction of other CVD risk factors, good control of hypertension and effective treatment of hyperlipidemia
- nutritional advice to reduce weight, lower saturated fat and avoid excess salt in the diet and to discourage the use of alcohol, particularly in individuals with hypertriglyceridemia.
- promotion of physical activity and exercise.

Hypertension is commonly associated with diabetes and may complicate. It is important independent risk factors for cardiovascular, renal, cerebral and peripheral vascular disease.

Hypertension should be detected early and treated aggressively if its contribution to increased morbidity and mortality in diabetes is to be avoided.(Alwan., 1994).

Guidelines for the management of hypertension in DM

- Unless the blood pressure is severely elevated, diagnosis should usually be based on high blood pressure (BP) measurements made under standard conditions on at least three occasions.
- Blood pressure is elevated when the BP is persistently >140 mmHg systolic and/or >90 mmHg diastolic.
- The presence of target-organ damage (e.g. retinal, renal or cardiovascular) should be evaluated.
- Other modifiable cardiovascular risk factors should be checked.
- In general, the goal of blood pressure treatment should be to maintain BP at <140 mmHg systolic and <85 mmHg diastolic.
- Treatment should initially be based on nonpharmacological therapy, namely weight reduction, dietary modification, increased physical activity and smoking cessation.
- The aim of dietary therapy should focus on a low salt intake (sodium intake of less than 100 mmol/day), and low saturated fat to reduce the risk of CVD. For overweight individuals, calorie reduction to achieve gradual weight loss should be planned together with regular physical exercise. Alcohol increases plasma triglyceride levels excessive consumption can also lead to a further rise in blood pressure.
- Drug treatment should be considered only if the therapy targets are not reached with nonpharmacological measures. An exception to this recommendation is severe hypertension (systolic of >180 or diastolic of >110) when drug treatment should be considered on presentation (Alwan ., 1994**).**

I.5.12.Treatment of DM

Categories of anti diabetic drugs :

I.5.12.1. Insulin

I.5.12.2. Insulin secretagogues
Increase release of insulin form pancrease and classified to

- **Sulfonylureas**
  eg : Glimepiride , Olipazide, Glyburide, Tolbutamide

§ Acute release of insulin by functioning beta cells of pancreatic islet tissue
§ Increase insulin sensitivity of liver, muscle, fat, RBCs, Monocytes

- **nonsulfonylurea hypoglycemic agents (metglitinides)**
  eg : Repaglinide, Nateglinide
- Enhances insulin secretion by functioning pancreas

I.5.12.3. Bigaunides
eg :Metformin hydrochloride

§ Reduces hepatic glucose overproduction
§ Increases insulin receptors
§ Decreased intestinal absorption of glucose

I .5.12.4. Thizolidediones
eg : Piglitazone, Rosiglitazone

§ Affects Liver, Muscle, and Fat
§ Decreases insulin resistance
§ Increases glucose uptake
§ Decreased hepatic glucose production
§ Affects PPARs (peroxizome proliferated activated receptors)creates liver side effects

I.5.12.5. Alpha-glucosidase inhibitor
eg : Acarbose, Miglitol
Block intestinal starch absorption

I. 5.12. 1. Insulin
Insulin used in treatment of type 1 diabetes in which the lack of insulin is the causative factor.The onset of action, peak effect and duration of action are determined by the insulin type and by the physical and chemical form of the insulin
.

I.5.12.1.A Classification of insulin preparations:.
Insulin preparations are classified according to onset and duration of action into the following groups:

I.5.12.1.A.a. Fast acting insulin
Conventional fast- acting insulin are soluble insulin (also known as neutral insulin). After subcutaneous injection the concentration rises to a peak after about 2 hours and then declines

over a further 4-8 hours. The fast acting recombinant insulin analogues (insulin lispro, insulin aspart and insulin glulisine) are more rapidly absorbed than the non-analogue soluble insulins and have a shorter duration of action. The analogues therefore offer more flexibility. They are more convenient for some patients as they can be given immediately before a meal rather than the 30 minutes before recommended for human soluble insulin. Another benefit is a reduced risk of hypoglycemia because of the shorter duration of action. These pharmacokinetic differences arise the short-acting analogues remain as monomers (single units) unlike regular soluble human insulin which self- associate into a hexameric(6-unit) form. Hexamers need to dissociate into dimers and monomers to be readily absorbed from subcutaneous.

I. 5. 12 .1.A.b. Intermediate-acting insulin

Conventional intermediate-acting insulin are insoluble, cloudy suspenions of insulin complexed with either protamine (also known as isophane or NPH insulin) or zinc (lente insulin). Over time, insulin dissociates from the protamine, which gives the preparation its extended activity. The onset of action is usually 1-2 hours with the peak effect being seen at 4-8 hours. There is considerable inter-patient variation in the duration of action, but it usually requires twice-daily administration to adequately cover a 24-hour period. Protamine insulin and soluble insulin do not interact when mixed together. As a result there is a wide range of ready-mixed(biphasic) preparations available containing varying proportions of isophane and soluble insulin.
Lente insulin is formed by producing a 30:70 mixture of an amorphous insulin and a crystalline zinc-insulin complex in suspension . It has a slower onset of action than isophane insulin and a longer duration of effect at the same dose. In order to maintain the integrity of the insulin crystals, all insulin zinc suspensions contain significant amounts of free zinc in solution. If mixed with soluble insulin, some of the latter may be precipitated into a loose complex if they remain in contact. Therefore, if these two insulins are mixed, they should be injected immediately.

I. 5. 12 .1.A.c. Long-acting insulin

More recently, long-acting insulin analogues such as insulin glargine and insulin detemir have action been developed using recombinant DNA technology. They both have a duration of of about 24 hours, a more predictable, flat profile of action with no pronounced peaks and less inter- and subject dosing variability (Roger and Cate.,2007). Insulin glargine It i s a long acting basal insulin analogue, given once daily. It consists of microcrystals that slowly release insulin, giving a long duration of action of 18 to 26 hours, with a "peakless" profile (according to the insulin glargine package insert). Pharmacokinetically, it resembles basal insulin secretion of non-diabetic pancreatic beta cells. Sometimes, in type 2 diabetes and in combination with a short acting sulfonylurea , it can offer moderate control of serum glucose levels. In the absence of endogenous insulin type 1 diabetes, depleted type 2 (in some cases) or latent autoimmune diabetes of adults in late stage insulin glargine needs the support of fast acting insulin taken with food to reduce the effect of prandially derived glucose .
Unlike some other longer-acting insulins, glargine must not be diluted or mixed with other insulin or solution in the same syringe (Kaplan ., 2004).

§ Insulin glargine

It is formulated at an acidic pH 4, where it is completely water soluble. After subcutaneous injection of the acidic solute (which can cause discomfort and a stinging sensation), when a physiologic pH (approximately 7.4) is achieved the increase in pH causes the insulin to come out of solution resulting in the formation of higher order aggregates of insulin hexamers. In the neutral subcutaneous space, higher-order aggregates form, resulting in a slow, peak less dissolution and absorption of insulin from the site of injection. It can achieve a peak less level for at least 24 hours.. The higher order aggregation slows the dissociation of the hexamers into insulin monomers, the functional and physiologically active unit of insulin. This gradual process ensures that small amounts of insulin glargine are released into the body continuously, giving an almost peakless profile (Bolli .,1999).

§ Insulin detemir

Insulin detemir has a long duration of action and is formulated at neutral PH. It differs from human insulin by omission of the amino acid threonine at position B30 and the attachment of a fatty acid chain (myristic acid) to lysine at position B29. The modification allows the insulin molecule to reversibly bind to albumin, via the fatty acid chain, following absorption from subcutaneous injection. This reduces the amount of free, active insulin detemir (bound insulin is inactive).

The long duration of action is produced by dissociation of the insulin molecule from albumin (Roger and Cate., 2007).

I.5.12.1.B. Adverse effects of insulin

1 - Hypoglycemia

2- Lipohypertrophy

Thickening of subcutaneous tissues can occur at injection sites because of recurrent injection in the same area. As well as looking unsightly, it can result in impaired and erratic insulin absorption, leading to poor glycemic control. The solution is to rotate injection sites. Localized skin reactions occasionally occur but resolve even with continued use of the same insulin preparation.

3-Systemic allergic reactions rarely occur with the current universal use of highly purified insulin (Roger and Cate., 2007).

I.5.12 .2. Insulin secretagogues

These are the drugs that increase Insulin output from Pancreas.

I.5.12.2.a. Sulfonylureas

Sulfonylureas were the first widely used oral anti-hyperglycaemic medications. They are *insulin secretagogues,* triggering insulin release by inhibiting the K_{ATP} channel of the pancreatic beta cells. The "second-generation" drugs are now more commonly used. They are more effective than first-generation drugs and have fewer side-effects, All may cause weight gain. Sulfonylureas bind strongly to plasma proteins. They are useful only in Type 2 diabetes, as they work by stimulating endogenous release of insulin. They work best with patients over 40

years old who have had diabetes mellitus for under ten years. They can be safely used with metformin or glitazones. The primary side effect is hypoglycemia.

Typical reductions in glycated hemoglobin (HbA1C) values for second-generation sulfonylureas are 1.0–2.0%.

- **First-generation agents**
  - tolbutamide
  - acetohexamide
  - tolazamide
  - chlorpropamide
- **Second-generation agents**
  - glipizide
  - glibenclamide
  - glimepiride
  - gliclazide
  - glycopyramide
  - gliquidone (Rendell., 2004).

I.5.12.2.b. Nonsulfonylurea secretagogues

Meglitinides help the pancreas produce insulin and are often called "short-acting secretagogues." They act on the same potassium channels as sulfonylureas, but at a different binding site. By closing the potassium channels of the pancreatic beta cells, they open the calcium channels, thereby enhancing insulin secretion. They are taken with or shortly before meals to boost the insulin response to each meal. If a meal is skipped, the medication is also skipped. Typical reductions in glycated hemoglobin (HbA1C) values are 0.5–1.0%.

- repaglinide , nateglinide

  Adverse reactions include weight gain and hypoglycemia (Rendell., 2004).

I.5.12.3. Biguanides

Biguanides reduce hepatic glucose output and increase uptake of glucose by the periphery, including skeletal muscle. Although it must be used with caution in patients with impaired liver or kidney function, metformin, a biguanide, has become the most commonly used agent for type 2 diabetes in children and teenagers. Among common diabetic drugs, metformin is the only widely used oral drug that does not cause weight gain.

Typical reduction in glycated hemoglobin (HAb1C) values for metformin is 1.5–2.0%

- Metformin may be the best choice for patients who also have heart failure (Eurich et a., 2007). but it should be temporarily discontinued before any radiographic procedure involving intravenous iodinated contrast, as patients are at an increased risk of lactic acidosis.
- Phenformin and Buformin was used from 1960s through 1980s, but were withdrawn due to lactic acidosis risk (Fimognari et al., 2006).

Metformin is usually the first-line medication used for treatment of type 2 diabetes. In general, it is prescribed at initial diagnosis in conjunction with exercise and weight loss, as opposed to in the past, where it was prescribed after diet and exercise had failed. There is an immediate release as well as an extended-release formulation, typically reserved for patients experiencing GI side-effects. It is also available in combination with other oral diabetic medications.

I.5.12.4. Thiazolidinediones

Thiazolidinediones (TZDs), also known as "glitazones," bind to PPARγ, a type of nuclear regulatory protein involved in transcription of genes regulating glucose and fat metabolism. These PPARs act on peroxysome proliferator responsive elements (PPRE). The PPREs influence insulin-sensitive genes, which enhance production of mRNAs of insulin-dependent enzymes. The final result is better use of glucose by the cells.

Typical reductions in glycated hemoglobin (HbA1c) values are 1.5–2.0%. Some examples are:

- rosiglitazone : the European Medicines Agency recommended in September 2010 that it be suspended from the EU market due to elevated cardiovascular risks.
- pioglitazone : may decrease the overall incidence of cardiac events in people with type 2 diabetes who have already had a heart attack.
- troglitazone : used in 1990s, withdrawn due to hepatitis and liver damage risk (Hinterthuer and Adam ., 2008).

I.5.12.5. Alpha- glucosidase inhibitors

They are not technically hypoglycemic agents because they do not have a direct effect on insulin secretion or sensitivity. These agents slow the digestion of starch in the small intestine, so that glucose from the starch of a meal enters the bloodstream more slowly, and can be matched more effectively by an impaired insulin response or sensitivity. These agents are effective by themselves only in the earliest stages of impaired glucose tolerance, but can be helpful in combination with other agents in type 2 diabetes.

Typical reductions in glycated hemoglobin (HbA1C) values are 0.5–1.0%.

eg :miglitol , acarbose, voglibose

These medications are rarely used in the United States because of the severity of their side-effects (flatulence and bloating). They are more commonly prescribed in Europe. They do have the potential to cause weight loss by lowering the amount of sugar metabolized.

Research has shown that the culinary mushroom maitake (Grifola frondosa) has a hypoglycemic effect,, possibly due to the mushroom acting as a natural alpha glucosidase inhibitor (Konno et al., 2001).

I.5.13. Other treatments of DM

I.5.13.1. Injectable Incretin mimetics

Incretins are insulin secretagogues. The two main candidate molecules that fulfill criteria for being an incretin are glucagon-like peptide-1(GLP-1) and gastric inhibitory peptide (glucose-dependent insulinotropic peptide, GIP). Both GLP-1 and GIP are rapidly inactivated by the enzyme dipeptidyl peptidase-4 (DPP-4).

Incretins- Risk of Pancreatitis. Recent studies Incretins are thought to cause inflammation that could lead to pancreatitis/pancreatic cancer (Michelle., 2012).

I.5.13.1.a. Injectable Glucagon-like peptide analogs and agonists

Glucagon-like peptide (GLP) agonists bind to a membrane GLP receptor.As a consequence, insulin release from the pancreatic beta cells is increased. Endogenous GLP has a half-life of only a few minutes, thus an analogue of GLP would not be practical.

eg : Exenatide. is the first GLP-1 agonist approved for the treatment of type 2 diabetes. Exenatide is not an analogue of GLP but rather a GLP agonist (Gallwitz., 2006).

Exenatide has only 53% homology with GLP, which increases its resistance to degradation by DPP-4 and extends its half-life.(Typical reductions in A1C values are 0.5–1.0%.

These agents may also cause a decrease in gastric motility, responsible for the common side-effect of nausea, and is probably the mechanism by which weight loss occurs (Cvertkovic., 2007).

I.5.13.1.b. Dipeptidyl Peptidase-4 Inhibitors

GLP-1 analogs resulted in weight loss and had more gastrointestinal side-effects, while in general DPP-4 inhibitors were weight-neutral and increased risk for infection and headache, but both classes appear to present an alternative to other antidiabetic drugs. However, weight gain and/or hypoglycaemia have been observed when DPP-4 inhibitors were used with sulfonylureas; effect on long-term health and morbidity rates are still unknown. Blood concentration of the incretin GLP-1 by inhibiting its degradation by dipeptidyl peptidase-4.

eg :vildagliptin ,sitagliptin,saxagliptin ,linagliptin ,allogliptin

DPP-4 inhibitors lowered hemoglobin A1C values by 0.74%, comparable to other antidiabetic drugs (Amori et al., 2007 and Doucet et al., 2011).

I.5.13.2. Injectable Amylin analogues

Amylin agonist analogues slow gastric emptying and suppress glucagon. They have all the incretins actions except stimulation of insulin secretion. As of 2007, pramlintide is the only clinically available amylin analogue. Like insulin, it is administered by subcutaneous injection. The most frequent and severe adverse effect of pramlintide is nausea, which occurs mostly at the beginning of treatment and gradually reduces. Typical reductions in A1C values are 0.5–1.0%.(Amori et al., 2007 and Doucet et al., 2011).

I.5.14. Metformin

Metformin is an oral antidiabetic drug in the biguanide class. It is the first-line drug of choice for the treatment of type 2 diabetes, in particular, in overweight and obese people and those with normal kidney function. Metformin mainly works by suppressing glucose production by the liver. Metformin is the only antidiabetic drug that has been conclusively shown to prevent the cardiovascular complications of diabetes. It helps reduce LDL cholesterol and triglyceride levels, and is not associated with weight gain. As of 2010, metformin is one of only two oral antidiabetics in the WHO model list of Essential Medicines (the other being glibenclamide).
First synthesized and found to reduce blood sugar in the 1920s, metformin was forgotten for the next two decades as research shifted to insulin and other antidiabetic drugs. Interest in metformin was rekindled in the late 1940s after several reports that it could reduce blood sugar levels in

people, and in 1957, French physician Jean Sterne published the first clinical trial of metformin as a treatment for diabetes. It was introduced to the United Kingdom in 1958, Canada in 1972, and the United States in 1995. Metformin is now believed to be the most widely prescribed antidiabetic drug in the world; in the United States alone, more than 48 million prescriptions were filled in 2010 for its generic formulations. (Bailey and Day., 2004).

I.5.14.1. METFORMIN AND SAFETY PROFILE

Gastrointestinal side effects, i.e. diarrhea, nausea, bloating and metallic taste in the palate are not uncommon when treatment with metformin is started, affecting 1%-30% of patients. Increasing the dose gradually, most side effects may be diminished. There is clear relationship between the dosage and effect of metformin, so the most effective dosage of metformin observed in studies was 2000 mg/day. Increasing the metformin dosage from 2000 to 3000 mg/day only reduced fasting blood glucose levels by further 5%, raising the incidence of gastrointestinal side effects. The risk of hypoglycemia was low. Lactic acidosis is the most dangerous side effect, fortunately rare, with an incidence of 0-0.084 cases/1000 patient years .To minimize the risk of lactic acidosis, contraindications should be observed, i.e. impaired renal function (limit value of creatinine clearance 60 mL/min), severe liver disease, pancreatitis, alcoholism, hypoxic states, respiratory insufficiency, severe cardiac insufficiency, cardiovascular shock, metabolic acidosis, diabetic ketoacidosis, low serum level of vitamin B12, preoperative, perioperative and postoperative states, radiological procedures using contrast, advanced age, and calorie restrictions (<1000 cal per day) (Andre ., 2010).

I.5.14.2. Mechanism of action of metformin

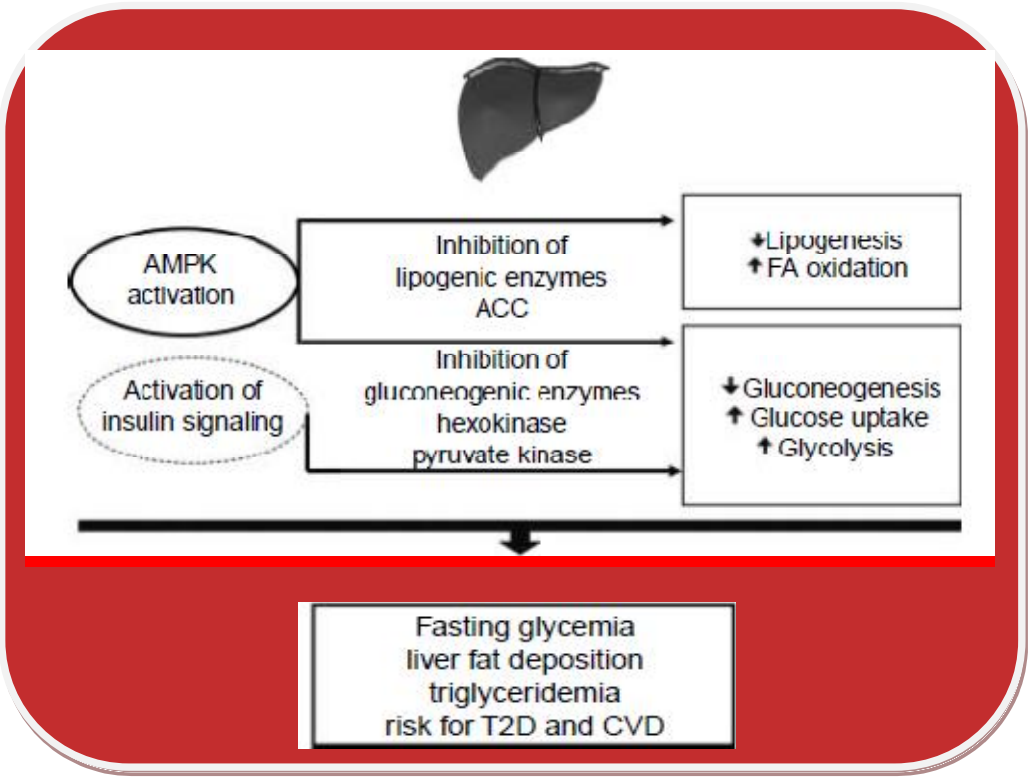
Metformin decreases hyperglycemia primarily by suppressing glucose production by the liver (hepatic gluconeogenesis)(Kirpichnikov et al., 2002). The "average" person with type 2 diabetes has three times the normal rate of gluconeogenesis; metformin treatment reduces this by over one third(Hundal et al., 2000). Metformin activates AMP-activated protein kinase (AMPK), an enzyme that plays an important role in insulin signaling, whole body energy balance, and the metabolism of glucose and fats;(Towler and Hardie.,2007). activation of AMPK is required for metformin's inhibitory effect on the production of glucose by liver cells (Zhou et al., 2001). The mechanism by which biguanides increase the activity of AMPK remains uncertain; however, research suggests that metformin increases the amount of cytosolic AMP(as opposed to a change in total AMP or total AMP/ATP) (Zhang .,2007). Metformin and other biguanides may antagonize the action of glucagon, thus reducing fasting glucose levels (Miller et al ., 2013).In addition to suppressing hepatic glucose production, metformin increases insulin sensitivity, enhances peripheral glucose uptake (by phosphorylating GLUT-4 enhancer factor), increases fatty acid oxidation, and decreases absorption of glucose from the gastrointestinal tract. Increased peripheral utilization of glucose may be due to improved insulin binding to insulin receptors (Collier et al ., 2006).

AMPK probably also plays a role, as metformin administration increases AMPK activity in skeletal muscle.(Musi et al.,2002) , AMPK is known to cause GLUT4deployment to the plasma membrane, resulting in insulin-independent glucose uptake. Some metabolic actions of metformin do appear to occur by AMPK-independent mechanisms (Saeedi et al .,2008) **.**

Metformin exerts its principal metabolic action and especially its glucoregulatory action upon the liver Interest in the therapeutic use of metformin has been sparked by the recognition of its pleiotropic actions on several tissues, which are affected by IR and/or hyperinsulinemia. Although the liver is the primary target organ, metformin acts also on skeletal muscles, adipose tissue (Evanthia and Charikleia .,2010).

- **Actions of metformin in the liver:**

Metformin exerts direct effects on hepatic glucose and lipid metabolism. Metformin suppresses gluconeogenesis mainly through AMPK dependent activation of key enzymes, whereas it enhances glucose uptake and glycolysis through the activation of hexokinase and pyruvate kinase. The enhancement of insulin signaling may play a part in the latter effect. In addition, metformin suppresses lipogenic enzymes, particularly acetyl-CoA carboxylase (ACC) activity via an AMPK-dependent pathway, thus leading to decreased lipogenesis but increased fatty acid oxidation. The net benefits of the above hepatic actions of metformin appear to be the decrease of fasting glucose and triglyceride levels and the diminution of liver fat content (Diagram 1).



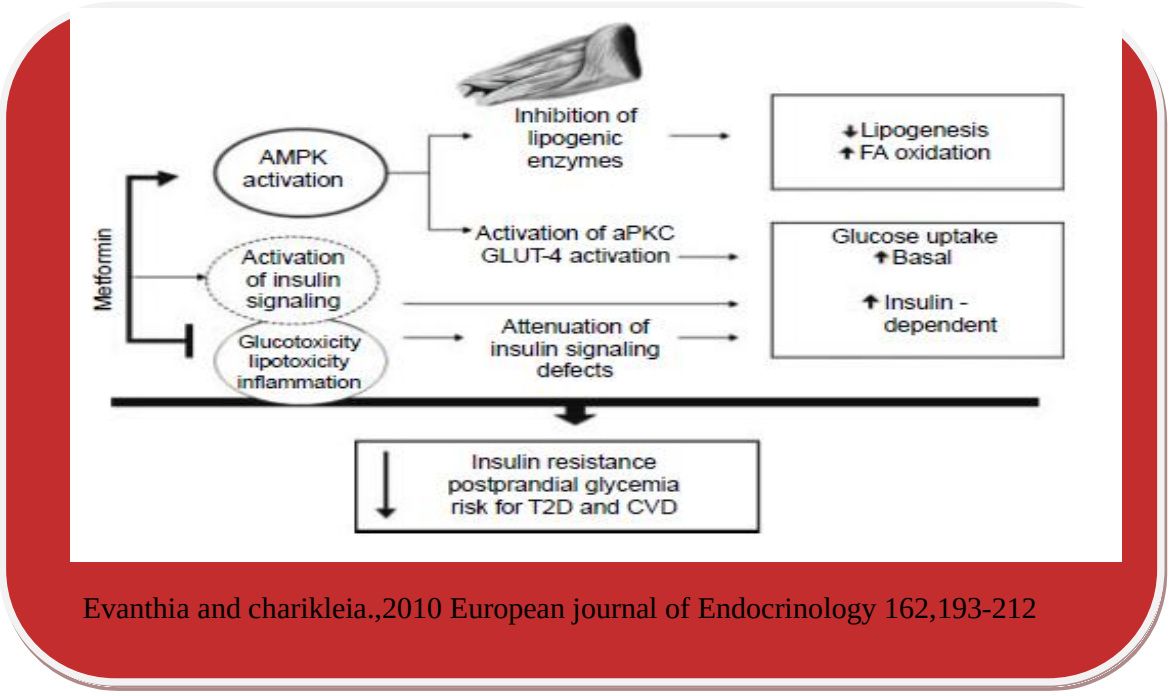
Evanthia and charikleia.,2010 European journal of Endocrinology 162,193-212

Diagram (1) Action of metformin in the liver

- **Actions of metformin on skeletal muscle:**

Metformin may moderately increase basal and insulin-stimulated glucose uptake. The former effect appears to be mediated by AMPK activation and subsequent aPKC and GLUT-4 activation, while the latter effect may involve the enhancement of insulin signaling either directly or indirectly through the attenuation of glucotoxicity, lipotoxicity, and inflammation. By activating AMPK, metformin may also inhibit lipogenesis, while enhancing fatty acid oxidation

in skeletal muscle. The net result of the above actions may be the attenuation of systemic insulin resistance and the decrease of postprandial glucose levels (Diagram 2).



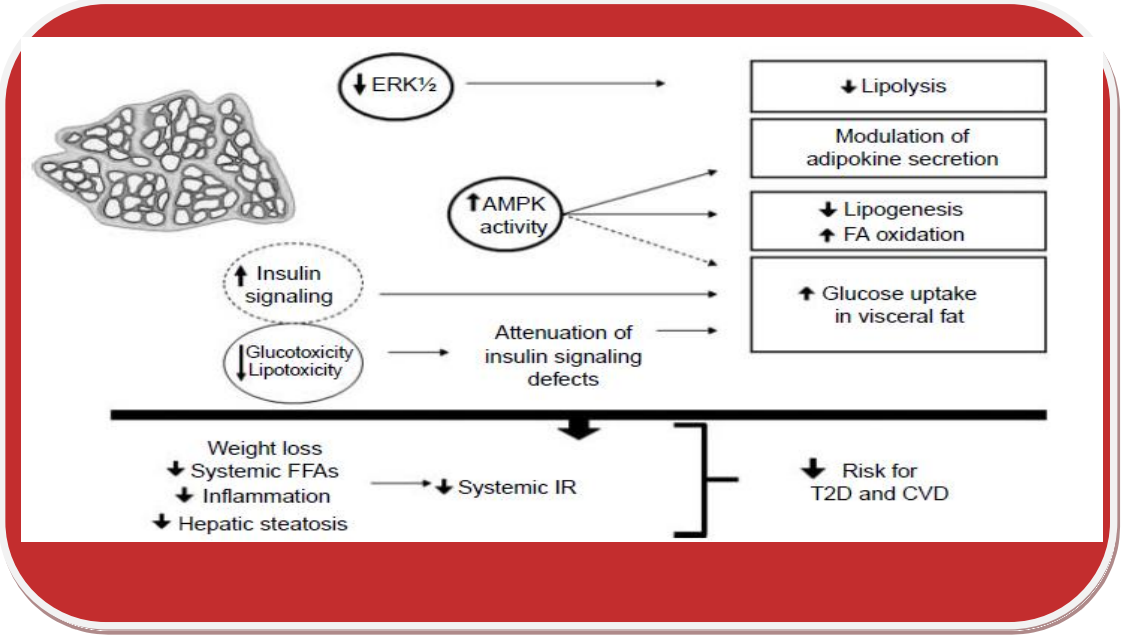
Evanthia and charikleia.,2010 European journal of Endocrinology 162,193-212

Diagram (2) Action of metformin in skeletal muscle

- **Actions of metformin on the adipose tissue:**

Metformin may inhibit agonist-induced lipolysis in adipocytes via inhibition of ERK1/2 phosphorylation, but it may counteract adipose tissue expansion through AMPK-dependent stimulation of FA oxidation and inhibition of lipogenesis in subcutaneous fat depot.

This antiadipogenic effect may contribute to reduced fat mass. The antilipolytic action of metformin could contribute to insulin sensitization through the decrease of systemic FFA levels. The contribution of metformin to the attenuation of glucotoxicity and lipotoxicity may further improve insulin sensitivity in adipose tissue. An AMPK-dependent mechanism may also enhance glucose uptake by visceral adipose tissue (Diagram 3) (Evanthia and Charikleia., 2010).



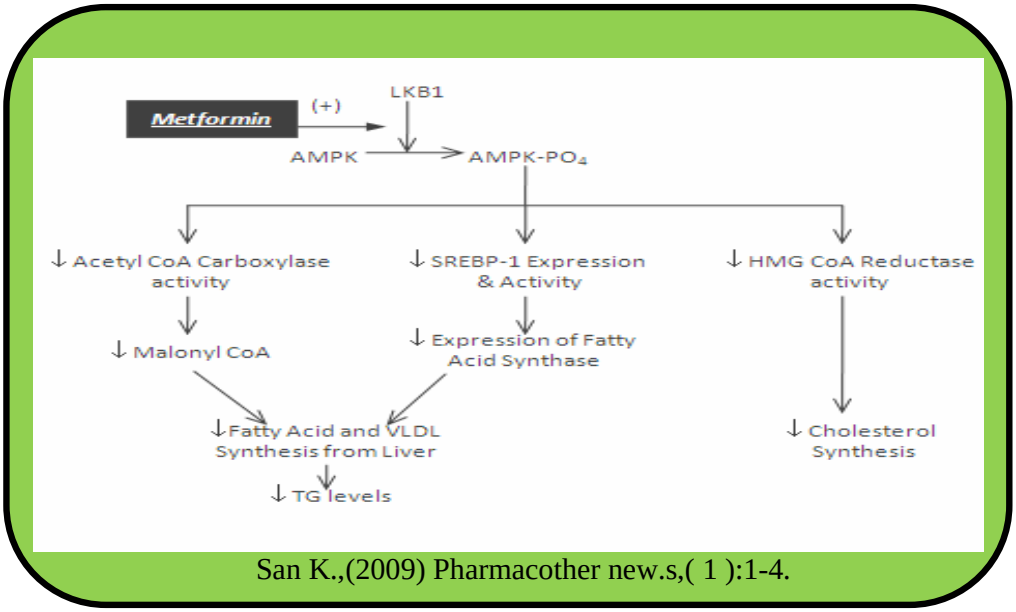
Evanthia and charikleia.,2010 European journal of Endocrinology 162,193-212

Diagram (3) Action of metformin on the adipose tissue

ACC= acetyl-CoA carboxylase AMPK= AMP-activated protein kinase;
FAs,= fatty acids; TG= triglycerides.

- **Mechanisms by which metformin improve lipid profile**

As shown in (diagram 4) the expected mechanism by which metformin improve lipid profile is that metformin phosphorylates and activates AMP-activated protein kinase (AMPK) which affect the transcription (production) of several key regulators of liver lipid production (lipogenesis) and hepatic gluconeogenesis (Zhou et al., 2001). The first regulation of lipogenesis is a reduction in the expression and activity of sterol regulatory element binding protein-1 (SREBP-1) which leads to two beneficial effects on lipids. One effect is a reduced expression of the enzyme fatty acid synthase, which leads to a reduction in fatty acid synthesis. These are both essential steps in the formation of triacylglycerols (also known as triglycerides (TG)) that make up the majority of VLDL being produced by the liver. Another effect is the phosphorylationof 3-hydroxy-3-methyl-glutary;-CoA reductase (HMG CoA Reductase) which reduces its cholesterol synthesis capabilities. The second regulation of lipogenesis is a phosphorylation of acetyl CoA carboxylase thereby inhibiting its activity. As a result, malonyl CoA levels are reduced leading to a reduction in fatty acid synthesis (need for TG production) and an enhancement of fatty acid oxidation (Clarke et al., 1990).



San K.,(2009) Pharmacother new.s,(1):1-4.

Diagram (4) Mechanisms of action of metformin in improvement of dyslipidemia

AMPK = AMP- activated protein kinase LKB_1 = an upstream primary kinase
SREBP = sterol regulatory element binding protein-I

I.5.14.3. Pharmacokinetics of metformin

1. Metformin has an oral bioavailability of 50–60% under fasting conditions, and is absorbed slowly. Peak plasma concentrations (C_{max}) are reached within one to three hours of taking immediate-release metformin and four to eight hours with extended-release formulations. The plasma protein binding of metformin is negligible, as reflected by its very high apparent volume of distribution . Steady state is usually reached in one or two days (Heller., 2007 and Bristol., 2008).
2. Metformin has low lipophilicity and, consequently, rapid passive diffusion of metformin through cell membranes is unlikely.Metformin is not metabolized. It is cleared from the body by tubular secretion and excreted unchanged in the urine. Metformin is undetectable in blood plasma within 24 hours of a single oral dose. The average elimination half-life in plasma is 6.2 hours. Metformin is distributed to (and appears to accumulate in) red blood cells, with a much longer elimination half-life: 17.6 hours (reported as ranging from 18.5 to 31.5 hours in a single-dose study of non-diabetic people) (Bristol., 2008 and Robert et al., 2003)

I.5.14.4. Medical uses of metformin

- **Type 2 diabetes**

The main use for metformin is in the treatment of diabetes mellitus type 2, especially in overweight people. In this group, over 10 years of treatment, metformin reduced diabetes complications and overall mortality by about 30% when compared with insulin and sulfonylureas and by about 40% when compared with the group only given dietary advice(UKPDS group., 1998). This difference held in people who were followed up for five to 10 years after the study (Holman et al., 2008). Since intensive glucose control with metformin appears to decrease the risk of diabetes-related endpoints in overweight people with diabetes,

and is associated with less weight gain and fewer hypoglycaemic attacks than are insulin and sulphonylureas, it may be the first-line pharmacological therapy of choice in this group. In addition, metformin had no effect on body weight: Over the 10-year treatment period, the metformin group gained about 1 kg, the same as the dietary advice group, while the sulfonylureas group gained 3 kg, and the insulin group, 6 kg (Selvin et al., 2008) As metformin affords a similar level of blood sugar control to insulin and sulfonylureas, it appears to decrease mortality primarily through decreasing heart attacks, strokes and other cardiovascular complications. Metformin has a lower risk of hypoglycemia than the sulfonylureas (Maharani ., 2010). although it has uncommonly occurred during intense exercise, calorie deficit, or when used with other agents to lower blood glucose,(Dipiro and Talbert et al.,2005). Metformin reduce LDL and triglyceride levels (Bolen et al.,2007).

- **Prediabetes**

Metformin treatment of people at risk for type 2 diabetes may decrease their chances of developing the disease, although intensive physical exercise and dieting work significantly better for this purpose.

- **Polycystic ovary syndrome (PCOS):**

Antidiabetic therapy has been proposed as a treatment for the PCOS, a condition frequently associated with insulin resistance, since the late 1980s (Kidson., 1998). The use of metformin in PCOS was first reported in 1994, in a small study conducted at the University of the Andes, Venezuela (Velazuez et al.,1994 and Teede., 2007). The United Kingdom's National Institute for Health and Clinical Excellence recommended in 2004 that women with PCOS and a body mass index above 25 be given metformin for anovulation and infertility. Another review recommended metformin unreservedly as a first-line treatment option because long-term metformin treatment has positive effects not only on anovulation, but also on insulin resistance, hirsutism, and obesity because its reduce serum androgen levels in these patients often associated with PCOS (Radosh. , 2009). Metformin has shown beneficial in reducing hyperinsulinaemia and hyperandrogenaemia in PCOS patients. Metformin improves insulin response during the oral glucose tolerance. Insulin sensitizers like metformin act directly on the thecal cells decreasing steroid production. concluded that metformin had direct inhibitory effect on androstenedione production in human ovarian thecal like androgen-producing tumor cells. Henceforth, these findings explains the mechanism for decrease in androgen levels with metformin (Pearce., 2009).

- **Gestational diabetes**

Several observational studies and randomized, controlled trials have found metformin is as effective and safe as insulin for the management of gestational diabetes (Tertti et al., 2008 and Rowan et al., 2008 and Nicholson et al., 2009). and a small case-control study has suggested the children of women given metformin instead of insulin may be healthier in the neonatal period (Balani et al ., 2009). and evidence on the long-term safety of metformin for both mother and child is still lacking (Cheung.,2009).

- **Prevention of weight gain**

A single randomized, controlled trial suggested metformin may reduce weight gain in people taking atypical antipsychotics, in particular, when combined with lifestyle interventions (education, dieting, and exercise) (Wu et al ., 2008).

- **Cancer prevention**

A large case-control study has suggested metformin may somewhat reduce the incidence of pancreatic cancer, whereas participants who had used insulin or secretagogues (such as the sulfonylureas) were found to have a higher risk of pancreatic cancer, compared to participants that had been treated with neither. The study had several limitations, however, and the reason for this risk reduction is still unclear (Li and Yeung et al ., 2009)**.**

Observational studies conducted by the University of Dundee have shown a decrease of 25–37% in cancer cases in diabetics taking metformin (Evans etal .,2005 and Libby et al., 2009) .

A direct action of metformin on cancer cells is suspected. Metformin exhibits a strong and consistent antiproliferative action on several cancer cell lines, including breast, colon, ovarian, pancreatic, lung and prostate cancer cells. These cellular studies were generally completed by preclinical studies showing a reliable antitumoral effect in various mouse models.
In addition, the first clinical trials demonstrated a beneficial effect in breast and colon cancer (Ben et al., 2010). Metformin is effective anti diabetic drug with a potential new indication for the management and chemoprevention in cancer.
Metformin activates AMPKinase by two separate mechanisms, the inhibition of oxidative phosphorylation/electron transport and resulting decrease in the ATP/AMP ratio and/or the direct activation of LKB1. Add-on to the inhibitory effects on protein synthesis – via inhibition of mTOR – the activation of AMPK may advance the generation of memory CD8 T lymphocytes and suppress cancer cachexia signals in the hypothalamus.
Inhibition of electron transport may be a lethal insult to cancer cells (diagram 5). Metformin shows increased memory CD8 T cells and in consequence it significantly improved the efficacy of an experimental anti-cancer vaccine (Pearce., 2009).

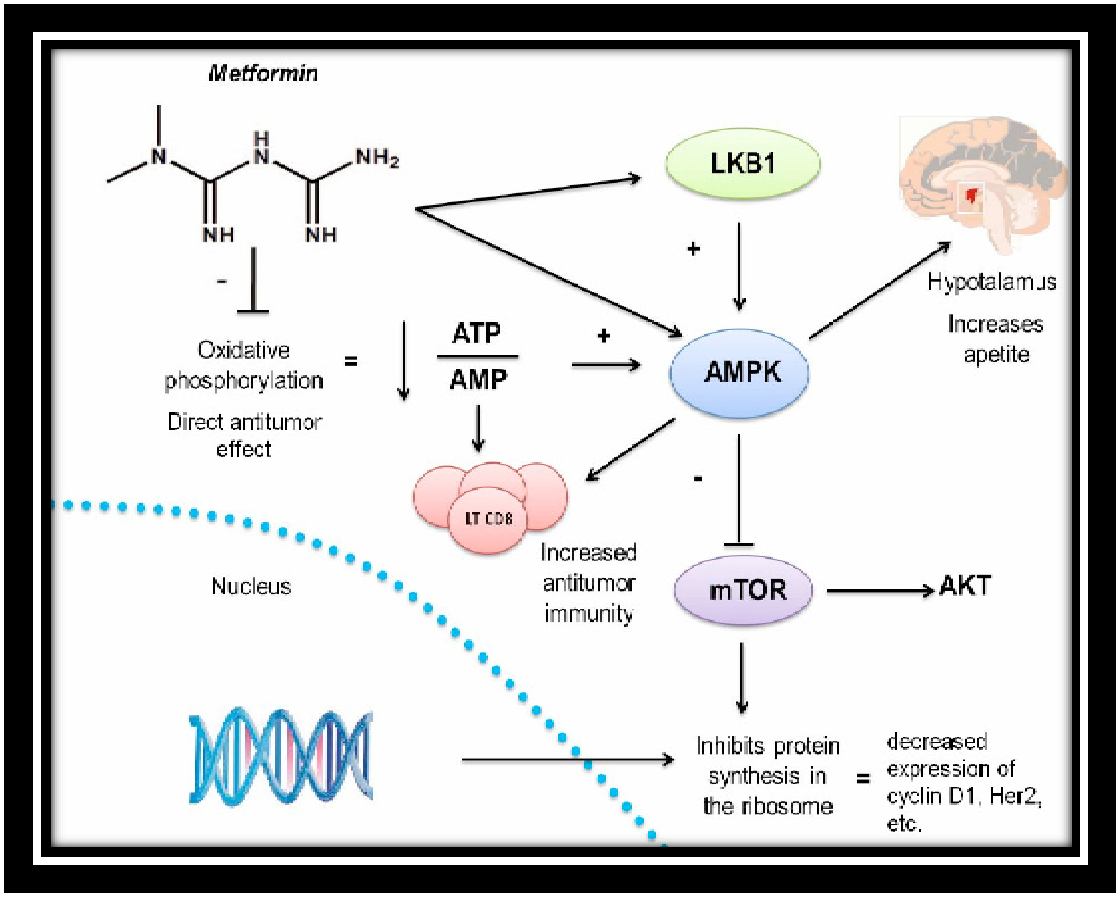


Diagram (5) Anti tumor action of metformin

pradeep and Sandip., 2012,International Journal of clinical cases ,4(1):5-12.

- **non-alcoholic fatty liver disease (NAFLD) (Marchesini and Brizi., 2001).**
- **premature puberty (Ibanez and Okn.g ., 2006).**

I.5.14.5. Adverse effects of metformin

1 - Gastrointestinal

Gastrointestinal upset, including diarrhea, cramps, nausea, vomiting and increased flatulence (Bolen et al., 2007) and it is most common when metformin is first administered, or when the dose is increased. The discomfort can often be avoided by beginning at a low dose (1 to 1.7 grams per day) and increasing the dose gradually. Gastrointestinal upset after prolonged, steady use is less common

2 - Lactic acidosis:

This complication is very rare, and the vast majority of these cases seem to be related to conditions, such as impaired liver or kidney function, rather than to the metformin itself (Khurana et al., 2010). The most serious potential adverse effect of biguanide use is lactic acidosis, the incidence for which is 9 per 100,000 person-years. Phenformin, another biguanide, was withdrawn from the market because of an increased risk of lactic acidosis (rate of 40-64 per 100,000 patient-years) (Stang et al ., 1999).

3 - Hormonal effects:

It decrease the blood levels of thyroid-stimulating hormone in people with hypothyroidism (Vigersky and Filmore et al.,2006). And in men testosterone.(Shegem and Nasir et al., 2002).

4 - Vitamin B_{12}deficiency

Long-term use of metformin has been associated with increased homocysteine levels, and mal absorption of vitamin B_{12}. Higher doses and prolonged use are associated with increased incidence of vitamin B_{12} deficiency, and some researchers recommend screening or prevention strategies (Ting and Szeto et al., 2006).

I.5.14.6. Contraindications of metformin

Metformin is contraindicated in people with any condition that could increase the risk of lactic acidosis, including kidney disorders (creatinine levels over 150 μmol/l (1.7 mg/dL), although this is an arbitrary limit), lung disease and liver disease. Unstable or acute congestive heart failure, increases risk of lactic acidosis with metformin in 2007 systematic review of controlled trials, however, suggested metformin is the only antidiabetic drug not associated with any measurable harm in people with heart failure, and that it may reduce mortality in comparison with other antidiabetic agents.(Eurich et al.,2007). Metformin is recommended to be discontinued before radiographic study involving iodinated contrast agents, (such as a contrast-enhanced CT scan or angiogram), as the contrast dye may temporarily impair kidney function, indirectly leading to lactic acidosis by causing retention of metformin in the body. Metformin can be resumed after two days, assuming kidney function is normal (Weir ., 1999 and Thomsen., 2003).

I.5.14.7. Interactions of metformin

The H_2-receptor antagonist cimetidine causes an increase in the plasma concentration of metformin, by reducing clearance of metformin by the kidneys both metformin and cimetidine are cleared from the body by tubular secretion, and both, particularly the cationic (positively charged) form of cimetidine, may compete for the same transport mechanism. A small double-blind, randomized study found the antibiotic cephalexin to also increase metformin concentrations by a similar mechanism; theoretically, other cationic medications may produce the same effect (Jayasagar et al., 2002).

I.5.15. **NEW approach in treatment of DM**

I.5.15.1. insulin therapy by Inhalation

In 2006 the U.S. Food and Drug Administration approved the use of Exubera, the first inhalable insulin. It was withdrawn from the market by its maker as of third quarter 2007, due to lack of acceptance. Inhaled insulin claimed to have similar efficacy to injected insulin, both in terms of controlling glucose levels and blood half-life. Currently, inhaled insulin is short acting and is typically taken before meals an injection of long-acting insulin at night is often still required (Nic .,2006). When patients were switched from injected to inhaled insulin, no significant difference was observed in Hb_{A1c} levels over three months. Accurate dosing was a particular problem, although patients showed no significant weight gain or pulmonary function decline over the length of the trial, when compared to the baseline (Cefalu et al.,2001). Following its commercial launch in 2005 in the UK, it was not as of July 2006) recommended by National Institute for Health and Clinical Excellence for routine use, except in cases where there is "proven injection phobia diagnosed by a psychiatrist or psychologist" (Nic ., 2006).

I.5.15. 2. Transdermal insulin

There are several methods for transdermal delivery of insulin. Pulsatile insulin uses micro jets to pulse insulin into the patient, mimicking the physiological secretions of insulin by the pancreas. Jet injection had different insulin delivery peaks and durations as compared to needle injection. Some diabetics find control possible with jet injectors, but not with hypodermic injection (Arora et al.,2007).

Both electricity using iontophoresis and ultrasound have been found to make the skin temporarily porous. The insulin administration aspect remains experimental, but the blood glucose test aspect of "wrist appliances" is commercially available (Dixit et al.,2007).

I.5.15.3 . Intranasal insulin

Intranasal insulin is being investigated. CPEX Pharmaceuticals reported phase 2a clinical trial preliminary results for its intranasal drug, Nasulin, on March 19, 2010. there's no word on when it might be expected on the market (Lalej et al., 2001).

I.5.15.4. Oral insulin

The basic appeal of oral hypoglycemic agents is that most people would prefer a pill to an injection. However, insulin is a protein, which is digested in the stomach and gut and in order to be effective at controlling blood sugar, cannot be taken orally in its current form.

Biopharmaceutical company called Biodel, Inc. is developing what it calls VIAtab, an oral formulation of insulin designed to be administered sublingually. This therapy is a tablet that dissolves in minutes when placed under the tongue. In a Phase I study, VIAtab delivered insulin to the blood stream quickly and resembled the first-phase insulin release spike found in healthy individuals. The company claims that an oral insulin therapy would be more convenient than currently available injectable or inhalable therapies, and they expect that convenience to result in increased insulin usage among the currently underserved early-stage patients with Type 2 diabetes, thus helping to create better long-term outcomes for that patient population.

Oramed Pharmaceuticals, Inc., a biotechnology company based n Jerusalem, Israel, is currently conducting Phase 2B clinical trials of its oral insulin capsule, ORMD-0801, on 30 patients diagnosed with type 2 diabetes.

An article published in the Journal of Diabetes Science and Technology indicates that Oramed's platform technology has two components:

1) A chemical make-up that protects insulin during passage through the gastrointestinal tract

2) Absorption enhancers so that insulin could be absorbed by the intestine. Oramed Pharmaceuticals, Inc. through Phase 1 clinical trials, has demonstrated that its oral insulin is safe, well tolerated, and has consistently reduced glucose and c-peptide levels in diabetic patients (Arbit and Kidron.,2009).
Australian biopharmaceutical company Apollo Life Sciences plans to enter the phase I trial of its oral insulin tablet in mid-2008 (Apollo.,2007).

I.5.15. 5. Pancreatic transplantation

Another improvement would be a transplantation of the pancreas or beta cell to avoid periodic insulin administration. This would result in a self-regulating insulin source. However, islet transplants had been highly experimental for many years, but some researchers in Alberta, Canada, have developed techniques with a high initial success rate (about 90% in one group). Nearly half of those who got an islet cell transplant were insulin-free one year after the operation; by the end of the second year that number drops to about one in seven. However, researchers at the University of Illinois at Chicago (UIC) have slightly modified the Edmonton Protocol procedure for islet cell transplantation and achieved insulin independence in diabetes patients with fewer but better-functioning pancreatic islet cells.(Gangemi et al., 2008). Longer-term studies are needed to validate whether it improves the rate of insulin-independence.
Beta cell transplant may become practical in the near future. Additionally, some researchers have explored the possibility of transplanting genetically engineered non-beta cells to secrete insulin (Zhu et al., 2004). Clinically testable results are far from realization at this time. Several other non-transplant methods of automatic insulin delivery are being developed in research labs, but none is close to clinical approval.

II.5.15.6. Artificial pancreas (Encapsulation approach)

A biological approach to the artificial pancreas is to implant bioengineered tissue containing islet cells, which would secrete the amounts of insulin, amylin and glucagon needed in response to sensed glucose. When islet cells have been transplanted via the Edmonton protocol, insulin production (and glycemic control) was restored, but at the expense of continued immunosuppression drugs. Encapsulation of the islet cells in a protective coating has been developed to block the immune response to transplanted cells, which relieves the burden of immunosuppression and benefits the longevity of the transplant one concept of the bio-artificial pancreas uses encapsulated islet cells to build an islet sheet which can be surgically implanted to function as an artificial pancreas. This islet sheet design consists of:

- An inner mesh of fibers to provide strength for the islet sheet;
- Islet cells, encapsulated to avoid triggering a proliferating immune response, adhered to the mesh fibers;
- A semi-permeable protective layer around the sheet, to allow the diffusion of nutrients and secreted hormones;
- A protective coating, to prevent a foreign body response resulting in a fibrotic reaction which walls off the sheet and causes failure of the islet cells.

Islet sheet with encapsulation research is pressing forward with large animal studies at the present, with plans for human clinical trials within a few years.
Clinical studies underway in New Zealand by Living Cell Technologies have encapsulated pig islet cells in a seaweed derived capsule. This approach has had very positive clinical studies and is currently underway in human trials as of 2008. So far, treatment using this method of cell encapsulation has been proven safe and effective and is the first to achieve insulin independence in human trials without immunosuppressant drugs.

I.5.15.7. Islet cell regeneration

Islet Neogenesis Associated Protein (INGAP) back in 1997. INGAP seems to be the product of a gene responsible for regenerating the islets that make insulin and other important hormones in the pancreas. (Yong et al., 2012).

I.5.15.8. Stem cells Implatation

Research is being done at several locations in which islet cells are developed from stem cells.
In January 2006, a team of South Korean scientists has grown pancreatic beta cells, which can help treat diabetes, from stem cells taken from the umbilical cord blood of newborn babies.
In 2007 it was the first study to use stem cell therapy in human diabetes mellitus This was initially tested in mice and there was the first publication of stem cell therapy to treat diabetes (Voltarelli et al., 2007)**.** Until 2009, there was 23 patients included and followed for a mean period of 29.8 months (ranging from 7 to 58 months). In the trial, severe immunosuppression with high doses of cyclophosphamide and anti-thymocyte globulin is used with the aim of "turning off" the immunologic system,and then autologous hematopoietic stem cells are rein fused to regenerate a new one. In summary it is a kind of "immunologic reset" that blocks the autoimmune attack against residual pancreatic insulin-producing cells. Until December 2009, 12 patients remained continuously insulin-free for periods ranging from 14 to 52 months and 8 patients became transiently insulin-free for periods ranging from 6 to 47 months. Of these last 8 patients, 2 became insulin-free again after the use of sitagliptin, a DPP-4 inhibitor approved only to treat type 2 diabetic patients and this is also the first study to document the use and complete insulin-independendce in humans with type 1 diabetes with this medication. In parallel with insulin suspension, indirect measures of endogenous insulin secretion revealed that it significantly increased in the whole group of patients, regardless the need of daily exogenous insulin use (Couri et al., 2009). In September 2008, scientists from the University of North Carolina at Chapel Hill School of Medicine have announced their success in transforming cells from human skin into cells that produce insulin. The skin cells were first transformed into stem cells and then had been differentiated into insulin-secreting cells.
In April 2006, the identification of novel stem cells from the umbilical cord blood with embryonic and hematopoietic characteristics was published (Yong and Honglan., 2006). Some months later the immune regulation of T lymphocytes by these stem cells was revealed (Yong et al., 2007). In 2009 the reversal of autoimmune-caused type 1 diabetes was confirmed in an animal experiment (Yong et al., 2009 and Zhao et al., 2010).
The following human clinical trial achieved an improvement of C-peptide levels, reduced the median glycated hemoglobin A1C (HbA1c) values, and decreased the median daily dose of insulin in both patient groups with and without residual beta cell function. The results of the phase I study of this Stem Cell Educator Therapy were published (Yong .,2012). Successful immune modulation by cord blood stem cells and the resulting clinical improvement in patient status may have important implications for other autoimmune and inflammation-related diseases without raising safety and ethical concerns.

I.5.15.9. Gene therapy

Technology for gene therapy is advancing rapidly such that there are multiple pathways possible to support endocrine function, with potential to practically cure diabetes.

- Gene therapy can be used to manufacture insulin directly an oral medication, consisting of viral vectors containing the insulin sequence, is digested and delivers its genes to the upper intestines. Those intestinal cells will then behave like any viral infected cell, and will reproduce the insulin protein. The virus can be controlled to infect only the cells which respond to the presence of glucose, such that insulin is produced only in the presence of high glucose levels.

Due to the limited numbers of vectors delivered, very few intestinal cells would actually be impacted and would die off naturally in a few days. Therefore by varying the amount of oral medication used, the amount of insulin created by gene therapy can be increased or decreased as needed. As the insulin-producing intestinal cells die off, they are boosted by additional oral medications.

- Gene therapy might eventually be used to cure the cause of beta cell destruction, thereby curing the new diabetes patient before the beta cell destruction is complete and irreversible.
- Gene therapy can be used to turn duodenum cells and duodenum adult stem cells into beta cells which produce insulin and amylin naturally. By delivering beta cell DNA to the intestine cells in the duodenum, a few intestine cells will turn into beta cells, and subsequently adult stem cells will develop into beta cells. This makes the supply of beta cells in the duodenum self replenishing, and the beta cells will produce insulin in proportional response to carbohydrates consumed (Stratta and Alloway., 1998).

I.5.15.10. Beta cell transplantation

Transplants of exogenous beta cells have been performed experimentally in both mice and humans, but this measure is not yet practical in regular clinical practice partly due to the limited number of beta cell donors. Thus far, like any such transplant, it has provoked an immune reaction and long-term immunosuppressive drugs have been needed to protect the transplanted tissue (Shapiro et al., 2006). An alternative technique has been proposed to place transplanted beta cells in a semi-permeable container, isolating and protecting them from the immune system. Stem cell research has also been suggested as a potential avenue for a cure since it may permit regrowth of Islet cells which are genetically part of the treated individual, thus perhaps eliminating the need for immuno-suppressants (Vinik et al., 2004).

I.5.15.11. Testosterone replacement thrapy

Testosterone replacement therapy may improve glucose tolerance and insulin sensitivity in diabetic hypogonadal men (Traish et al., 2009). Moreover testosterone may have a protective effect on pancreatic beta cells, which is possibly exerted by androgen-receptor-mediated mechanisms and influence of inflammatory cytokines (Zitzmann., 2009).

I.5.15.12. Invokana (canagliflozin)

SGLT2 (sodium glucose co-transporter) inhibitor,
reducing renal glucose reabsorption and increasing
urinary glucose excretion
Used as monotherapy or adjunct in Type 2 Diabetes
Dose: 100-300mg daily
Avoid in Cr Cl < 30ml/min
HbA1c reductions: 0.9-1.2%
Adverse Events: urinary tract infections, hypotension, mild hypoglycemia (Michelle ., 2012).

I.5.16. Statins

Statins is hydroxyl methyl glutaryl - CoA reductase inhibitor they (HMG-CoA) are a class of drugs used to lower cholesterol levels by inhibiting the enzyme HMG-CoA reductase, which plays a central role in the production of cholesterol in the liver. Increased cholesterol levels have been associated with cardiovascular disease, and statins have been found to prevent cardiovascular disease in those who are at high risk.

I.5.16.a. Mechanism of action statins

Statins act by competitively inhibiting HMG-CoA reductase, the first committed enzyme of the HMG-CoA reductase pathway. Because statins are similar to HMG-CoA on a molecular level, they take the place of HMG-CoA in the enzyme and inhibit HMG-CoA reductase, so block the pathway for synthesizing cholesterol in the liver. This is significant because most circulating cholesterol comes from internal manu, facture rather than the diet. When the liver can no longer produce cholesterol, levels of cholesterol in the blood will fall. Cholesterol synthesis appears to occur mostly at night,(Miettinen.,1982) so statins with short half-lives are usually taken at night to maximize their effect. Studies have shown greater LDL and total cholesterol reductions in the short-acting simvastatin taken at night rather than the morning,(Saito et al.,1991). but have shown no difference in the long-acting atorvastatin (Cilla et al., 1996) statin also Increase LDL uptake in hepatocytes (liver cells) sense the reduced levels of liver cholesterol and seek to compensate by synthesizing LDL receptors to draw cholesterol out of the circulation. This is accomplished via protease enzymes that cleave a protein called "membrane-bound sterol regulatory element binding protein", which migrates to the nucleus and causes increased production of various other proteins and enzymes, including the LDL receptor. The LDL receptor then relocates to the liver cell membrane and binds to passing LDL and VLDL particles (the "bad cholesterol" linked to disease). LDL and VLDL are drawn out of circulation into the liver, where the cholesterol is reprocessed into bile salts. These are excreted, and subsequently recycled mostly by an internal bile salt circulation, (Ma et al.,1986). LDL-lowering potency varies between agents. Cerivastatin is the most potent, (withdrawn from the market in August, 2001 due to risk of serious rhabdomyolysis) followed by (in order of decreasing potency), rosuvastatin, atorvastatin, simvastatin, lovastatin, pravastatin, and fluvastatin(Shepherd et al., 2003) .The relative potency of pitavastatin has not yet been fully established.

I.5.16.b. Adverse effects of statins

The most common adverse side effects are raised liver enzymes and muscle problems. Other possible adverse effects include cognitive loss, neuropathy, pancreatic and hepatic dysfunction, and sexual dysfunction (Golomb and Evans ., 2008).

Some patients on statin therapy report myalgias,muscle cramps, or, less frequently, gastrointestinal or other symptoms. Liver enzyme derangements, generally return to normal either without discontinuance over time or after briefly discontinuing the drug (Gaist et al., 2002).

- **Myositis and myopathy**

Rare reactions include myositis and myopathy, with the potential for rhabdomyolysis (the pathological breakdown of skeletal muscle) leading to acute renal failure (Graham et al., 2004). The risk was over 10-fold greater if cerivastatin was used, or if the standard statins (atorvastatin, fluvastatin, lovastatin, pravastatin, or simvastatin) were combined with fibrate (fenofibrate or gemfibrozil) treatment. Cerivastatin was withdrawn by its manufacturer in 2001.

The risk of myopathy was suggested to be lowest with pravastatin and fluvastatin, probably because they are more hydrophilic and as a result have less muscle penetration. Lovastatin induces the expression of gene atrogin-1, which is believed to be responsible in promoting muscle fiber damage (Hanai et al., 2007).

- **Hyperglycemia**

Statins may slightly increase the risk of hyperglycemia with higher doses appearing to have a larger effect (Preiss et al., 2011).

I.5.16.c. Other effects of statins

Statins exhibit action beyond lipid-lowering activity in the prevention of atherosclerosis. direct ultrasound evidence of atheroma regression during statin therapy. Researchers hypothesize that statins prevent cardiovascular disease via four proposed mechanisms

1- Improve endothelial function

2- Modulate inflammatory responses

3- Maintain plaque stability

4- Prevent thrombus formation (Nissen et al., 2006).

Aim of the study

The objective of the present study are:

1- To investigate the efficacy and safety of metformin at two different doses in the treatment of newly diagnosed type 2 diabetic patients as drug of first choice.

2-To study the efficacy and safety of chronic use of metformin in type 2 diabetic patients.

3-To elucidate the efficacy and safety of combination therapy of insulin and metformin in the treatment of type 2 diabetic patients.

4- To compare the efficacy of (metformin) and (insulin + metformin), versus, (insulin + metformin + simvastatin) in improvement of diabetes associated dyslipidemia in type 2 diabetic patients.

5- To collerate the dose of simvastatin with its lipid lowering efficacy.

CHAPTER- II

PATIENTS AND METHOD

II.1. Study design:

The record study was designed as unicenter study and was conducted at Benghazi Diabetic Centre and the protocol was approved by the local research committee of Faculty of Medicine, Benghazi University.

All the participants of the study gave informed consent to the doctor for using their blood parameters data determined during the course of treatment at the center for this purpose .

The protocol of study was designed as:

II.1.A - Prospective observational study

This part of study include the newly diagnosed type 2 DM patients

II.1.B - Retrospective study

This part included three categories of type 2 DM patients who were subjected to different regimen of antidiabetic drugs and have disease history for more than 2 years.

For all groups of type 2 patients the efficacy and safety of antidiabetic drugs were evaluated.

II.2. Patients:

A total of 160 Libyan type 2 DM patients were enrolled in the study and selected for a follow up on the basis of inclusion and exclusion criteria

The number of males and females were 97 and 63 respectively. Patients were eligible to participate in the study was aged between 40-60 years and if they had glycated hemoglobin $\geq$ 7%

All patients were interviewed for their past medication history, concomitant diseases before their participation in the study.

Patients name ,geneder, age, file number, date of visit were recorded

The exclusion criteria included were:

Type 2 DM patients, pregnancy, breast feeding ,acute MI, CHF, renal diseases, liver diseases like acute hepatitis , ketoacidosis ,patients taking lipid lowering agents except for the last group of patients. Patients who did not respond to treatment or who subjected to change treatment regimen during the period of study were also excluded.

The patients groups were divided to the following:

1- Newly diagnosed type 2 DM patients, for whom the physician decided to prescribe metformin in doses of (1g - 2g/day).

2-Type 2 DM patients treated with metformin for more than two years.

3-Type 2 DM patients undergoing combination therapy of insulin& metformin for more than two years.

4-Type 2 DM patients undergoing combination therapy of insulin& metformin & simvastatin for more than one year.

II. 3. Data collection:

1- First part of study(prospective study)

These were the newly diagnosed type 2 DM patients for whom physician decided to prescribe metformin in a dose of (1g or 2g/day),the duration of the study was three months, starting from the recruitment day (individual baseline), till the second visit (three months from the individual baseline).

Patients name, file number, age, geneder, weight(Kg), height (cm), date of visit were recorded, all patients were subjected to the following investigations:

* Fasting blood sugar values

* 2hr-postprandial blood sugar values

* Glycated hemoglobin(HbA1c%)

* Lipid profile values, (Total cholesterol (TC), Triglyceride(TG), High density lipoprotein(HDL-C), Low density lipoprotein(LDL-C).

* Liver function tests (ALT, AST, ALP, Bilirubin)

* Renal function tests (Urea, creatinine)

All these investigation were done at the biochemistry laboratory of the diabetic center .

The investigation results were recorded and kept for the next visit.

All patients were subjected to follow up for the next three months and after 12 weeks the same investigations were repeated for all patients. And results recorded again.

II.4. Efficacy and Safety evaluation:

II .4.1. Efficacy outcome:

Patients were observed for improvement of the primary efficacy variables (FBG, PPBG ,HbA1C reduction, body mass index(BMI), reduction, lipid profile& atherogenic index(AI), value reduction), at the end period of study relative to the baseline.

Body mass index (**BMI**),was calculated by the following equation:

BMI = WT(kg)/Hight(m2) ,(Keys.,1972).

Atherogenic index(**AI**), was calculated by the following equation:

AI = TC(mg/dl)/HDL-C(mg/dl) .(Rahman.,2013)

II.4.2 Safety outcome:

Safety was assessed on the basis of adverse events reported during the study and it was measured by the effect of drugs on patients renal function and hepatic function.

2- second part of study (retrospective study):

To assess the efficacy and safety of the investigated anti diabetic drugs
which were prescribed for more than 2 years and these included the following patients groups.

1-Type 2 DM patients taking metformin alone as monotherapy in a dose of (1g to 2g/day).
2-Type 2 DM patients taking metformin in combination to insulin.
3-Type 2 DM patients taking combination therapy of insulin &metformin.& simvastatin 40mg).

The insulin used was human insulin (mixtard 30/70) ,and it was administered as a mixture of short acting insulin(30%), and long acting insulin (70%) in a dose of (30-60 units).
All patients were interviewed for diabetes and antidiabetic drugs history and their names, ages , geneder, were recorded.
The other investigated parameters:

- FBG , PPBG , HbA1C
- Lipid profile (TC, TG, HDL-C, LDL-c)
- LFT (ALT, AST, ALP, Bilrubin)
- RFT (Creatinin, Urea)
- AI was calculated by the same equation .

The parameters of different treated groups were compared to determine the efficacies and safety of each drug regimen.

II.2.5. Statistical analysis of data:

The results were expressed as the mean ± S.E.M and the data were assessed by the method of analysis of variance (ANOVA). if this analysis indicated significant difference among the groups means then the multiple comparisons between by POST HOC test (LSD), or paired sample test.

A P value of ≤ 0.05 was considered statistically significant, P value of $\leq 0.01$was considered statistically highly significant, and $P \leq 0.001$ was considered very high significant.

CHAPTER- III

RESULTS

III.1. Metabolic effects of different doses (1g&2g/ day) of metformin in type 2 diabetic patients

III. 1.1. Effect of metformin (1g/day) on body weight and BMI of type 2 diabetic after 12 weeks treatment

By using the paired sample test and as shown in table (3) and fig (1).The result indicated that there was significant decrease ($P < 0.026$), in body weight of treated patient as compared to their body weight at the start of study.

The result presented in table (3) and fig (2), also showed that there was a very highly significant reduction ($P < 0.001$), in BMI of treated diabetic patients as compared to their BMI at the start of study.

III. 1.2. Effect of metformin (2g/day) on body weight and BMI of type 2 diabetic patient after 12 weeks of treatment.

Data presented in the table (3) and fig (1), indicated that the body weight of treated patients was very highly significant decreased ($P < 0.001$) as compared with initial weight at the start of study.

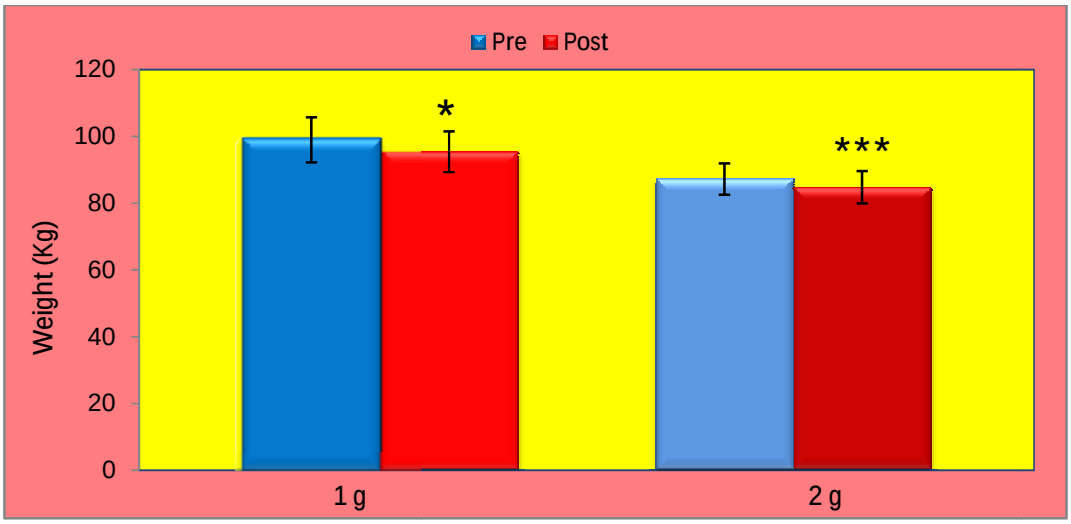
The result also indicated that there was very highly significant reduction ($P < 0.007$) in BMI value of treated group as compared to their BMI at the start of study as showed in table(3) and fig(2). Because of data are very high homogenous the significant reduction on BW &BMI were not appear very clear in histogram.

Table 3: Effect of different doses of metformin (1g & 2g/day) on body weight& BMI of type 2 diabetic patients after 12 weeks of treatment.

	Metformin (1g/day)			Metformin(2g/day)		
	Pre	Post	P-value	Pre	Post	P-value
Body weight (kg)	99.0±6.7	*95.4±6.1	0.02	87.2± 4.6	***84.7± 4.8	0.001
BMI(kg/m^2)	36.0± 2.7	***35.4±2.6	0.001	32.2± 1.8	**30.9± 1.9	0.007

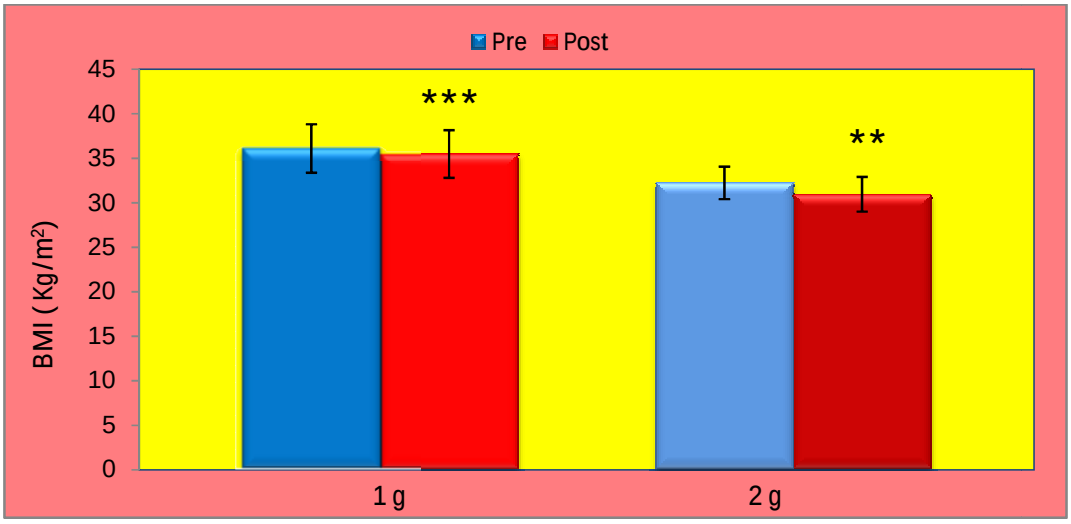
Pre = pre-treatment Post = post-treatment
The data are expressed as mean ± SEM
*** = significantly decreased in post treated as compared to pre treated patients**
**** = highly significant decreased in post treated as compared to pre treated patients**
***** = very highly significant decreased in post treated as compared to pre treated patients**

Fig 1 : Effect of different doses of metformin (1g & 2g/day) on body weight of type 2 diabetic patients after 12 weeks of treatment



Results expressed as mean ± SEM
Pre= pre treatment Post= post treatment
*** significant decreased in post treated patients as compared to pre treated patients**
***** very highly significant decreased in post treated patients as compared to pre treated patients**

Fig 2 : Effect of different doses of metformin (1g & 2g/day) on BMI of type 2 diabetic patients after 12 weeks of treatments



Results expressed as mean ± SEM
Pre = pre treatment Post = post treatment BMI = body mass index
**** significant decreased in post treated patients as compared to pre treated patients**
***** very highly significant decreased in post treated patients as compared to pre treated patients**

III.1. 3. Effect of metformin(1g/day) on glycemic control of type 2 diabetic patients after 12 weeks of treatment

AS shown in table(4) and fig (3,4), FBG and PPBG were highly significant decreased in treated patients as compared to their FBG and PPBG at the start of study ($P < 0.011$) and, ($P < 0.010$) respectively.

The result presented in table (4) and fig (5) showed that the level of HbA1c was significantly decreased ($P < 0.048$) in the treated diabetics as compared to their HbA1c at start of study.

III.1.4. Effect of metformin (2g/day) on glycemic control of type 2 diabetic patient.

As indicated in the table (4) and fig (3,4), the level of FBG of treated patient was highly significant decrease ($P < 0.013$), and PPBG level showed a very highly significant decrease ($P < 0.001$) as compared with their levels at the start of study.

As showed in the table (4) and fig (5), the level of HBA1c was highly significant decreased , ($P < 0.008$) in treated patient as compared it is to HbA1c value at the start of study.

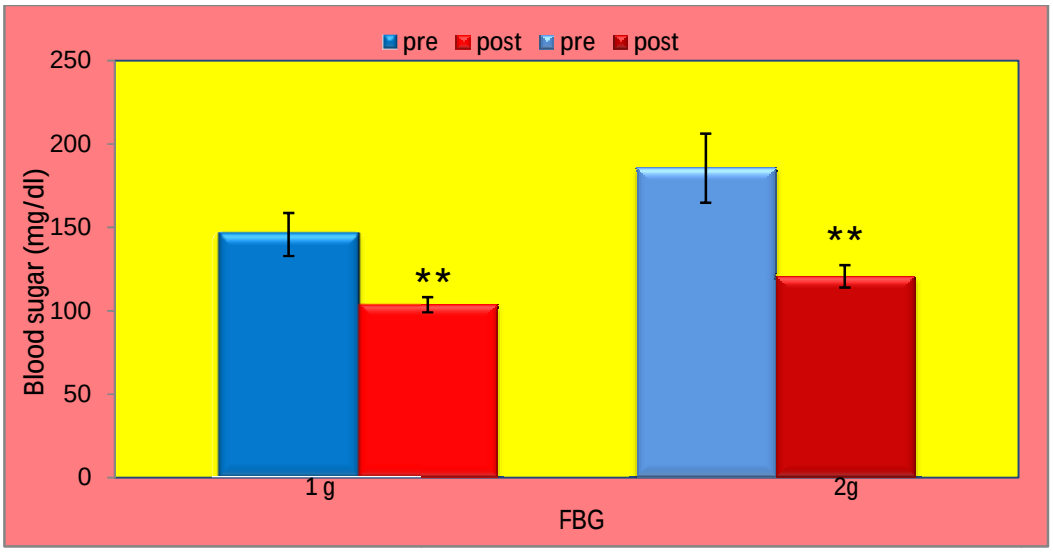
Table 4: Effect of different doses of metformin (1g&2g/day) on glycemic control of type 2 diabetic patients after 12 weeks of treatment.

	Metformin (1g/day)			Metformin (2g/day)		
	Pre	Post	P-value	Pre	Post	P-value
FBG(mg/dl)	145.8 ± 12.8	**103± 4.5	0.01	185.6± 20.7	**120.7±6.6	0.013
PPBG(mg/dl)	196.± 21.3	**143.7± 9.4	0.01	228.0± 18.8	***159.3±16.4	0.001
HbA1c (%)	7.3± 0.43	*6.6± 0.42	0.048	9.9± 0.99	**8.1±0.67	0.008

The data are expressed as mean ± SEM

*** = Significant decreased in post treated as compared to pre treated patients**
****= highly significant decreased in post treated as compared to pre treated patients.**
*****= very highly significant decreased in post treated as compared to pre treated patients.**

Fig 3: Effect of different doses of metformin (1g & 2g/day) on FBG of type 2 diabetic patients after 12 weeks of treatment

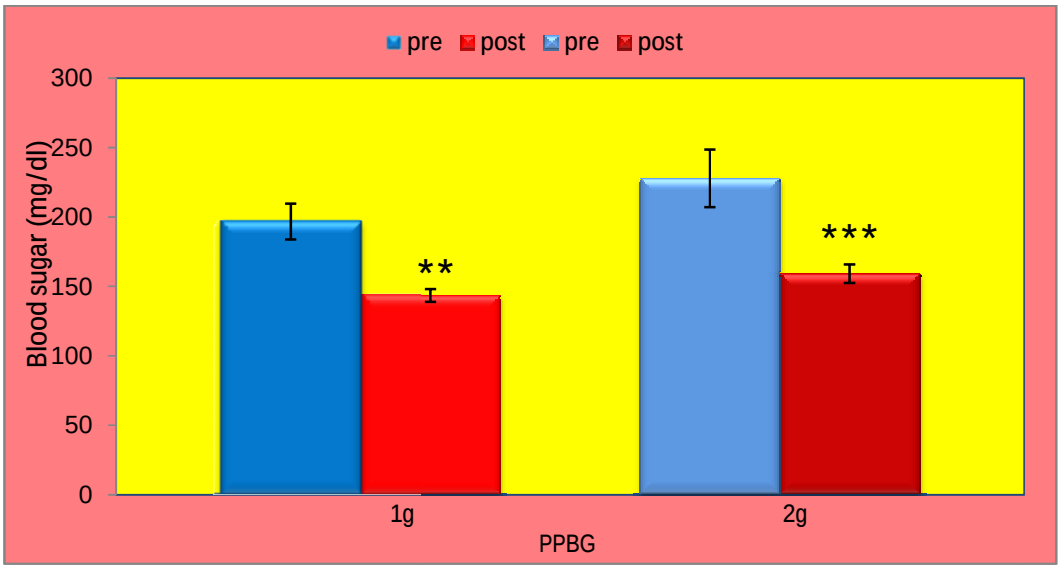


Results expressed as mean ± SEM

Pre= pre treatment Post= post treatment FBC= fasting blood sugar

*** * highly significant decreased in post treated patients as compared to pre treated patients.**

Fig 4: Effect of different doses of metformin (1g & 2g/day) on PPBG of type 2 diabetic patients after 12 weeks of treatment



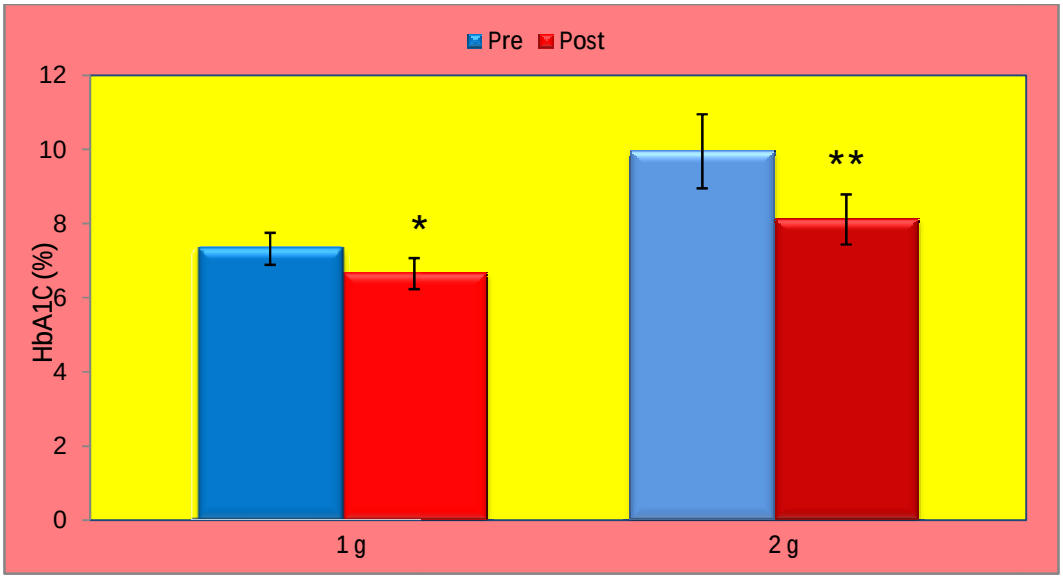
Results expressed as mean ± SEM

Pre= pre treatment Post= post treatment PPBG =post prandial blood sugar

** highly significant decreased in post treated patients as compared to pre treated patients.

*** very highly significant decreased in post treated patients as compared to pre treated patients.

Fig 5 : Effect of different doses of metformin (1g & 2g/day) on HbA1c of type 2 diabetic patients after 12 weeks of treatment



Results expressed as mean ± SEM

Pre= pre treatment Post= post treatment HbA1C = glycated hemoglobin

* significant decreased in post treated patients as compared to pre treated patients

** highly significant decreased in post treated patients as compared to pre treated patients

III.1.5. Effect of metformin (1g/day) on lipid profile of type 2 diabetic patients after 12 weeks of treatment

According to the result presented in the table (5) and fig (6), The levels of TC and TG, HDL-C, LDL-C of the treated diabetics showed no significant change ($P = 0.1$), ($P = 0.203$) ,($P = 0.598$),($P = 0.135$). respectively as compared to TC, TG, HDL-C,LDL-C values before treatment.

The results presented in the table (5) and fig (7), showed that the AI value was not significantly changed ($p = 0.857$) after 12 week of treatment as compared to the AI value at the start of study.

III.1.6. Effect of metformin (2g/day) on lipid profile of type 2 diabetic patients.

Results presented in the table (5) and fig (6), showed that the level of TC, was significantly decreased ($P < 0.027$), in the treated patient as compared with TC level at the start of study.

And there was significant decreased ($P < 0.028$),in level of Triglyceride of treated patients as compared to TG level at the start of study. Whereas the HDL-C level showed non significant increase ($P = 0.243$),in the treated patients as compared to patients at the start of study. Data also showed the level of LDL-C of treated diabetics was highly significant decreased ($P < 0.012$), as compared to their LDL-C values at the start of study. As showed in table (5) and fig (7), AI value was significant decreased ($P < 0.033$), in treated patient as compared to their AI value at the start of study.

Table 5: Effect of different doses of metformin (1g&2g/day) on lipid profile and AI of Type 2 diabetic patients after 12 weeks of treatment.

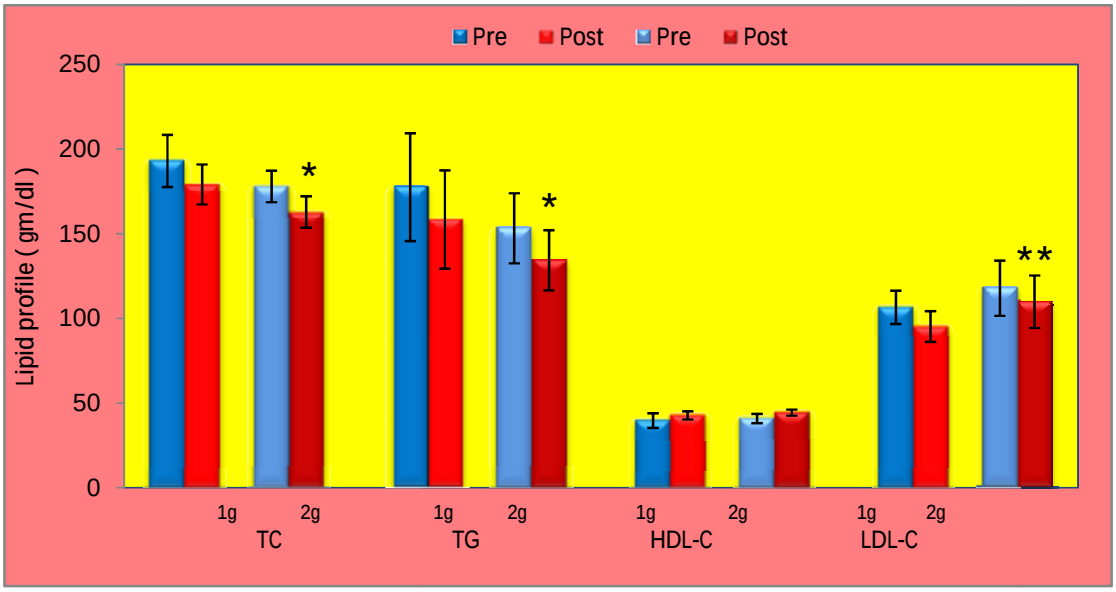
	Metformin (1g)			Metformin (2g)		
	Pre	Post	P-value	Pre	Post	P-value
TC(mg/dl)	193.2±15.4	179.3±11.8	0.1	178.1±9.3	*163.0±8.4	0.027
TG(mg/dl)	177.7±31.8	158.6±29.0	0.2	153.4±20.7	*134.5±17.7	0.028
HDL-C (mg/dl)	39.8±4.3	42.8±2.3	0.59	41.0±2.8	44.5±1.8	0.243
LDL-C (mg/dl)	106.7±9.8	95.4±9.1	0.13	118.0±16.3	**110±15.5	0.012
AI	4.3±0.62	4.2±0.22	0.85	4.0±0.39	*3.2±0.3	0.033

the data are expressed as mean ± SEM

* = significantly decreased as compared post with pre treated patient

**= highly significant decreased as compared post with pre treated patient

Fig 6 : Effect of different doses of metformin (1g & 2g/day) on lipid profile of type 2 diabetic patients after 12 weeks of treatment



Results expressed as mean ± SEM

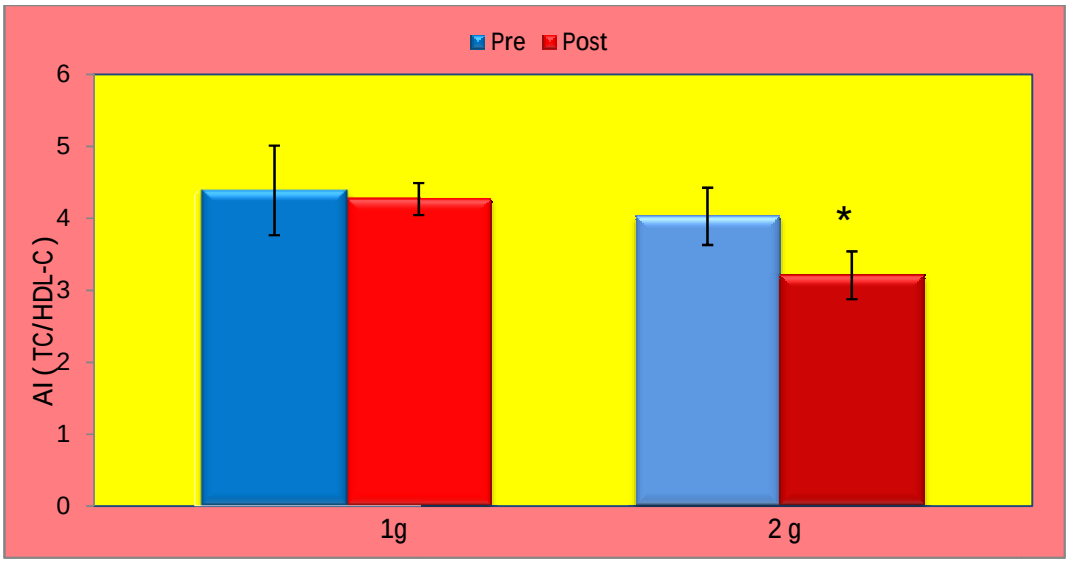
Pre = pre treatment Post = post treatment TC =Total cholesterol TG = Triglyceride

HDL-C = High density lipoprotein cholesterol, LDL-C = Low density lipoprotein cholesterol

* significant decreased in post treated patients as compared to pre treated patients

** highly significant decreased in post treated patients as compared to pre treated patients

Fig 7 : Effect of different doses of metformin (1g & 2g/day) on AI of type 2 diabetic patients after 12 weeks of treatment



Results expressed as mean ± SEM
Pre = pre treatment Post = post treatment AI=Atherogenic index
Results expressed as mean ± SEM
* significant decreased in post treated patients as compared to pre treated patients

III. 1.7. Effect of metformin(1g/day) on liver functions of type 2 diabetic patients after 12 weeks of treatment

The data presented in the table (6) fig (8), showed that the levels of ALT, AST, ALP enzymes in the treated patients show no significant change (P = 0.969) ,(P = 0.856), (P=0.38), respectively as compared to their levels at the start of study.
Result also showed no significant change (P=1.00) in bilirubin level of treated patients pre and post 12 weeks of treatment.

III.1.8. Effect of metformin (2g/day) on liver functions of type 2 diabetic patients after 12 weeks of treatment

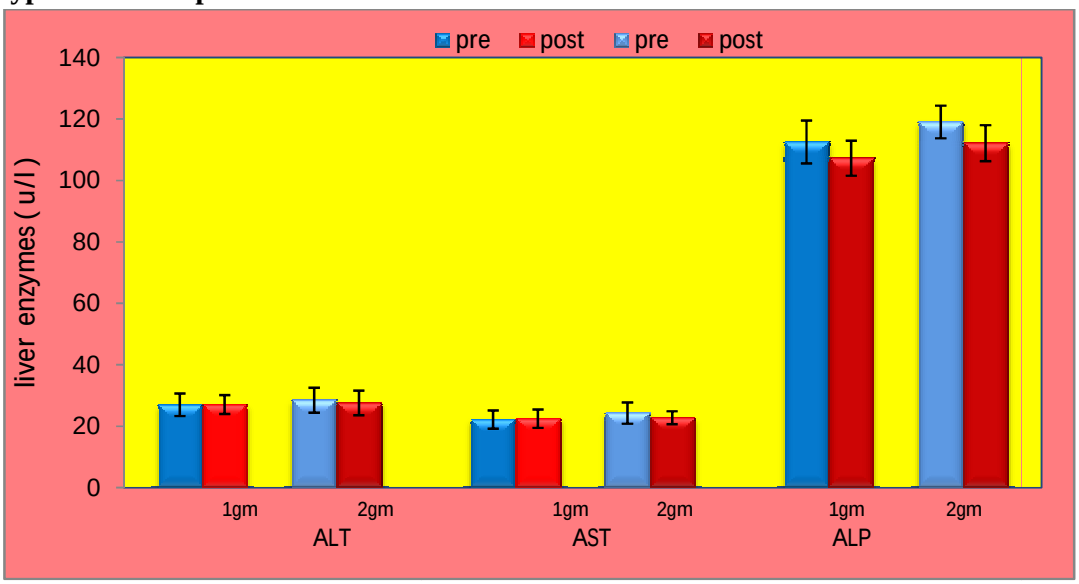
As results presented in the table (6) and fig (8),the levels of ALT, AST, of treated patients showed no significant change (P = 0.159), (P = 0.476) respectively, As compared with ALT, AST levels the start of study. the data showed there was non significant reduction (P < 0.084) in ALP level as compared to its level at the start of study.
Also the results revealed no significant change (P = 0.678) in bilirubin level of treated patient as compared with its level at the start of study.

Table 6: Effect of different doses of metformin (1g&2g/day) on liver parameters of type diabetic patients after 12 weeks of treatment.

	Metformin (1g/day)			Metformin (2g/day)		
	Pre	Post	P-value	Pre	Post	P-value
ALT(U/L)	27.0±3.6	27.1±3.0	0.96	28.5±4.0	27.6±3.5	0.159
AST(U/L)	22.2±2.9	22.5±3.0	0.85	24.3±3.4	22.8±2.1	0.476
ALP(U/L)	112.6±6.9	107.3±5.7	0.38	119.1±5.3	112.2±5.8	0.084
Bilirubin(mg/dl)	0.45±0.05	0.45±0.42	1.0	0.55±0.11	0.54±0.11	0.678

The data are expressed as mean ± SEM

Fig 8 : Effect of different doses of metformin (1g & 2g/day) on liver enzymes of type 2 diabetic patients after 12 weeks of treatment



Results expressed as mean ± SEM

Pre = pre treatment Post = post treatment

Results expressed as mean ± SEM

ALT= Alanine amino transferas AST= Aspartate amino transferase ALP= Alkaline phosphatase

NO significant change in post treated patients as compared to pre treated patients.

III. 1.9. Effect of metformin(1g/day) on renal functions of type 2 diabetic patients after 12 weeks of treatment

As shown in table (7) & fig (9) there was no significant change in the values of urea and creatinine (P =1.000)& (P = 0.28) of treated patients as compared to their values at the start of treatment.

III.1.10. Effect of metformin (2g/day) on renal functions of type 2 diabetic patients after 12 weeks of treatment

As shown in table (7) & fig (9) there was no significant change in the values of urea & creatinine (P = 0.845)&(P = 0.832) of treated patients as compared to their values at the start of treatment.

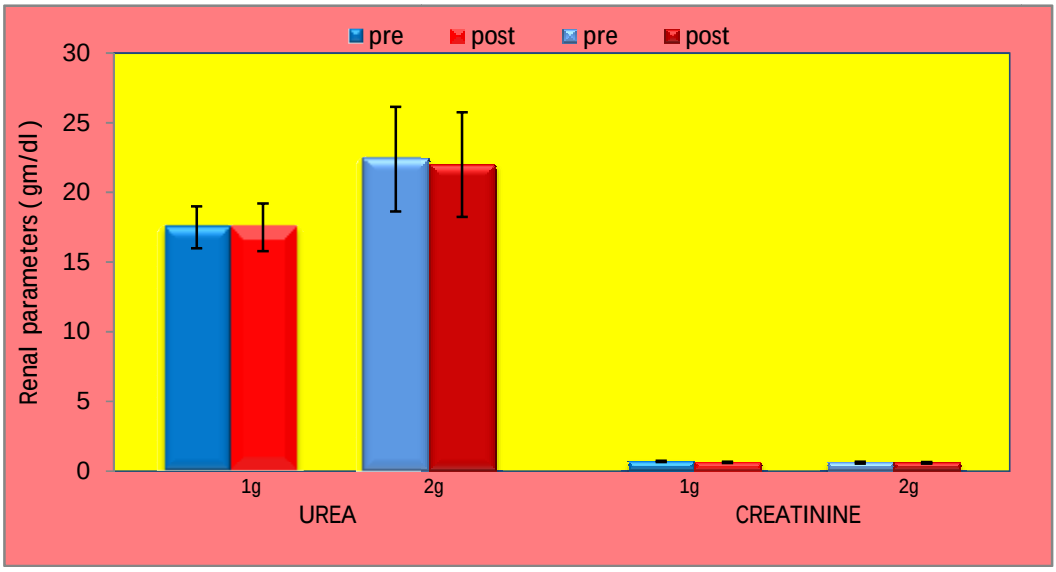
Table 7: Effect of different doses of metformin (1g&2g/day) on renal functions of type 2 diabetic patients after 12 weeks of treatment.

	Metformin (1g)			Metformin (2g)		
	Pre	Post	P-value	Pre	Post	P-value
Urea(mg/dl)	17.5±1.5	17.5±1.7	1.0	22.4±3.7	22.0±2.8	0.84
CT(mg/dl)	0.7±0.05	0.64±0.04	0.27	0.6±0.07	0.6±0.07	0.83

The data are expressed as mean ± SEM

No significant (NS) change as compared post with pre treated

Fig 9: Effect of different doses of metformin (1g&1g/day) on renal functions of type 2 diabetic patients after 12 weeks of treatment



Results are expressed as mean ± SEM

Pre= pre treatment Post= post treatment

NO significant change in post treated patients as compared to pre treated patients.

III.2. Metabolic effects of metformin alone as monotherapy and in combination with insulin and simvastatin on type 2 diabetic patients

III.2.1. Effects three drug regimens on glycemic control of type 2 diabetic patients.

In this study, by one way ANOVA test followed by Post Hoc tests (LSD). Data Presented in the table (8) and fig (10), showed no significant change (P = 0.432), in the level of FBG of the treated patients while the level of PPBG showed significant decrease, (P = 0.033), in patients treated by metformin+insulin as compared to patients treated by metformin alone.

The result also showed no significant change (p = 0.440) in level of PBG in patients treated by insulin+ metformin as compared to metformin+ Insulin +simvastatin treated group

Result in the table(8) and fig (11), showed very highly significant decreased (P < 0.001) in the level of HbA1c in metformin+insulin treated group as compared to metformin treated group ,the data also revealed no significant change (P = O.351), in the level of HbA1c level of metformin+insulin treated group as compared to metformin +insulin+simvastatin treated group.

Table 8: Effect of metformin as monotherapy and in combination with insulin and simvastatin on glycemic control of type 2 diabetic patients.

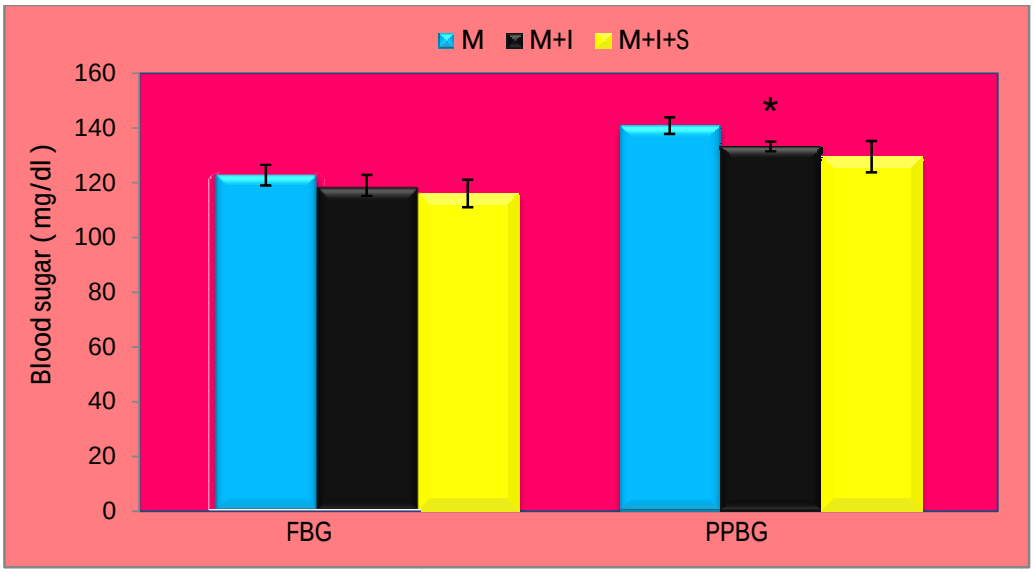
	FBG(mg/d)	PPBG(mg/dl)	HbAIC(%)
Metformin	122.8±3.7 P = 0.43	140.8±3.0	7.4±.0.08
Metformin+insulin	117.8±2.6 P = 0.43	*133.2±1.7 P = 0.03	**7.0±0.06 P = 0.001
Metformin+insulin+simvaststin	116.0±4.6 P = 0.43	129.5±5.7 P = 0.44	7.18±0.15 P =0.35

The data are expressed as mean ± SEM

*** = significant decreased as compared to metformin treated patients.**

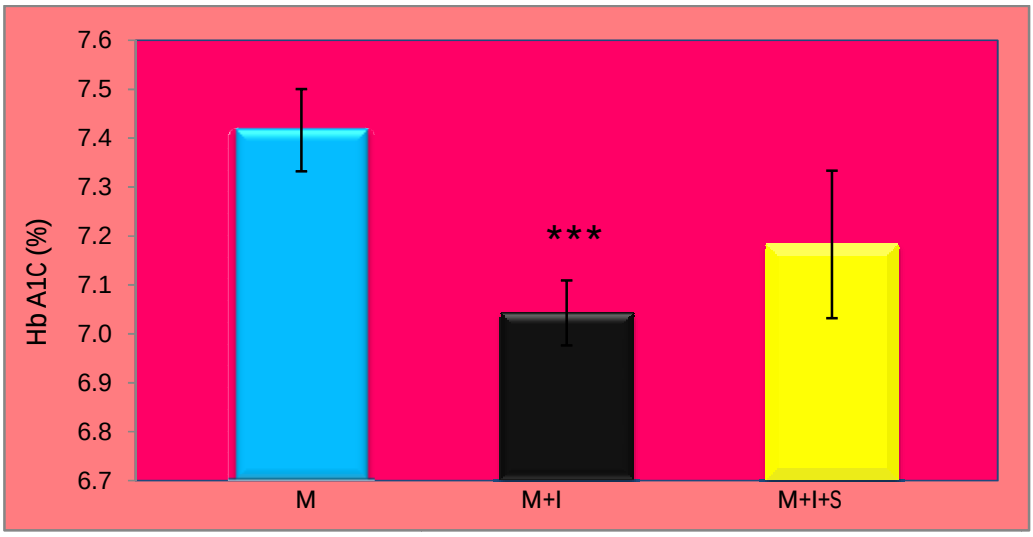
**** = highly significant decreased as compared to metformin treated patients.**

Fig 10 : Effect of metformin as monotherapy and in combination with insulin and simvastatin on glycemic control of type 2 diabetic patients



Results expressed as mean ± SEM
FBG = fasting blood sugar **PPBG = post prandial blood sugar**
M = metformin **I = insulin** **S = simvastatin**
* significant decreased as compared to metformin treated patients.

Fig 11: Effect of metformin as monotherapy and in combination with insulin and simvastatin on HbA1c level of type 2 diabetic patients



Results expressed as mean ± SEM
M = metformin I = insulin S = simvastatin
*** very highly significant decreased as compared to metformin treated patients

III. 2. 2. Effects of three drug regimens on lipid profile of type 2 diabetic patients.

III.2.2.a. Effect on total cholesterol:

The result showed compared to metformin treated group, metformin+insulin treated group showed no significant change (P = 0.259), in the level of TC . but there was very highly significant reduction (P < 0.00), in the TC level of the metformin+insulin+ simvastatin, Treated group as compared to metformin+insulin treated group. As showed in the table (9) and fig (12)

III.2.2.b. The effect on TG:

The result showed no significant change (P = 0.140) in the level of triglycerid of all three groups.

III.2.2.c Effect on HDL-C:

The level HDL-C showed non significant increase (P < 0.079), in patients treated by combined therapy of metformin+insulin as compared to metformin treated patients.
The data also revealed a significant increase (P < 0.021), in the HDL-C level in metformin+insulin+simvastastin treated group as compared to group treated by metformin+insulin.

III.2.2.d. Effect on LDL-C:

the result showed that there was no significant change (P = 0.794), in LDL-C level of metformin+insulin treated group as compared to metformin treated group.
While level of LDL-C was significantly decreased(P= 0.00),in metformin+ insulin+ simvastatin treated group as compared to Metformin + insulin treated group.

III.2.2.e. Effect on AI:

The Statistical analysis and result in table(9) and fig (13), indicated that there was no significant change (P = 0.445), in AI value of metformin+ insulin treated group as compared to metformin treated group.
While the Atherogenic index (AI) showed very highly significantly decreased
(P = 0.00), in patients treated by metformin + insulin + simvastatin as compared to patients treated by metformin + insulin. As showed in the table (9) and fig (12)

Table 9: Effect of metformin as monotherapy and in combination with insulin and simvastatin on lipid profile of Type2 diabetic patients.

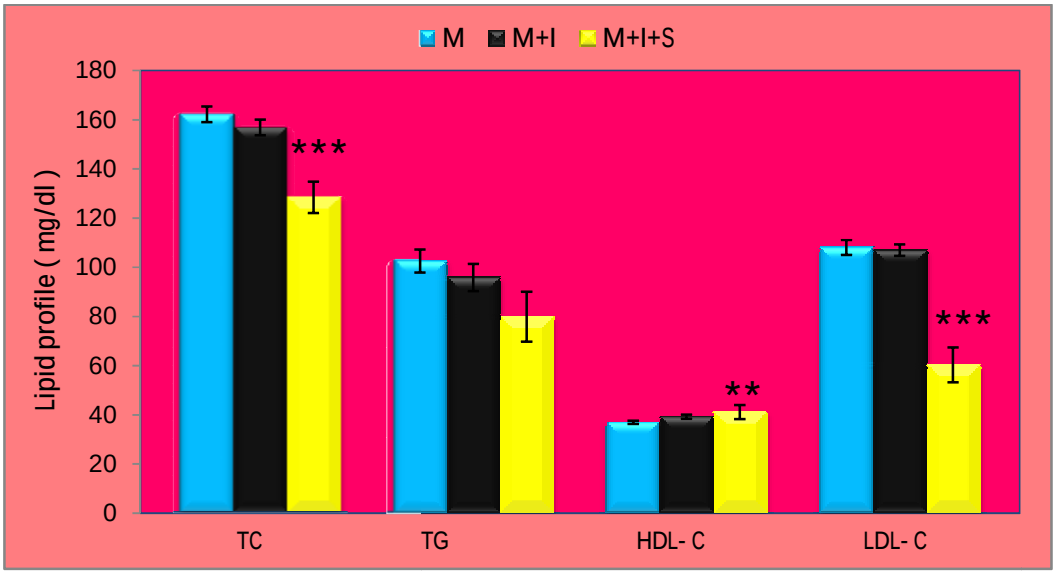
	TC(mg/dl)	TG(mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	AI=TC/HDL-C
Metformin	162.2±3.1	102.6±4.6 P = 0.14	37.0±0.6	108.1±3.0	4.38±0.12
Metformin+insulin	156.8±3.1 P = 0.25	95.9±5.5 P = 0.14	39.3±0.8 P = 0.07	107.0±2.3 P = 0.79	4.22±0.12 P = 0.44
Metformin+insulin + simvaststin	***128.5±6.3 p = 0.00	80.0±10.1 P = 0.14	*41.2±2.8 P = 0.02	***60.42 P = 0.00	***3.29±0.27 p=0.00

The data are expressed as mean ± SEM

*** = significant increased as compared to insulin + metformin treated patients**

***** = very highly significant decreased as compared to insulin + metformin treated patients**

Fig 12: Effect of metformin as monotherapy and in combination with insulin and simvastatin on lipid profile of type 2 diabetic patients.



Results expressed as mean ± SEM

M = metformin **I = insulin** **S = simvastatin**

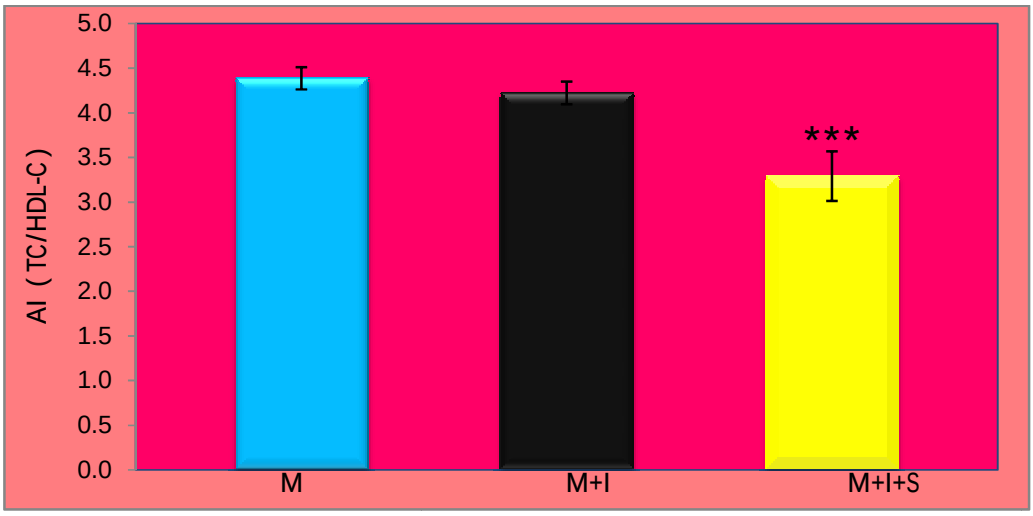
TC = Total cholesterol TG= Triglyceride HDL-C= High density lipoprotein

LDL-C= Low density lipoprotein

***** very highly significant decreased as compared to metformin+ insulin treated patients**

**** highly significantly increased as compared to metformin + insulin treated patient**

Fig 13: Effect of metformin as monotherapy and in combination with insulin and simvastatin on AI of type 2 diabetic patients.



Results expressed as mean ± SEM

M = metformin **I= insulin** **S = simvastatin** AI=Atherogenic index

***** very highly significant decreased as compared to metformin + insulin treated patients**

III.2.3. Effects of three drug regimes on liver functions of type 2 diabetic patients.

The data presented in the table (10) and fig (14) and , indicated that there was no significant change in the levels of ALT,AST and ALP of metformin + insulin treated patients as compared to metformin treated group with (P = 0.364 , P = 0.112 , P = 0.441) respectively. As well as no significant change in their levels in metformin+insulin+simvastatin treated group as compared to metformin+insulin treated group. With regard to bilirubin The data indicated that the level of bilirubin was highly significantly increased, (P<0.006) in metformin+insulin+simvastatin treated group as compared to the two other groups.

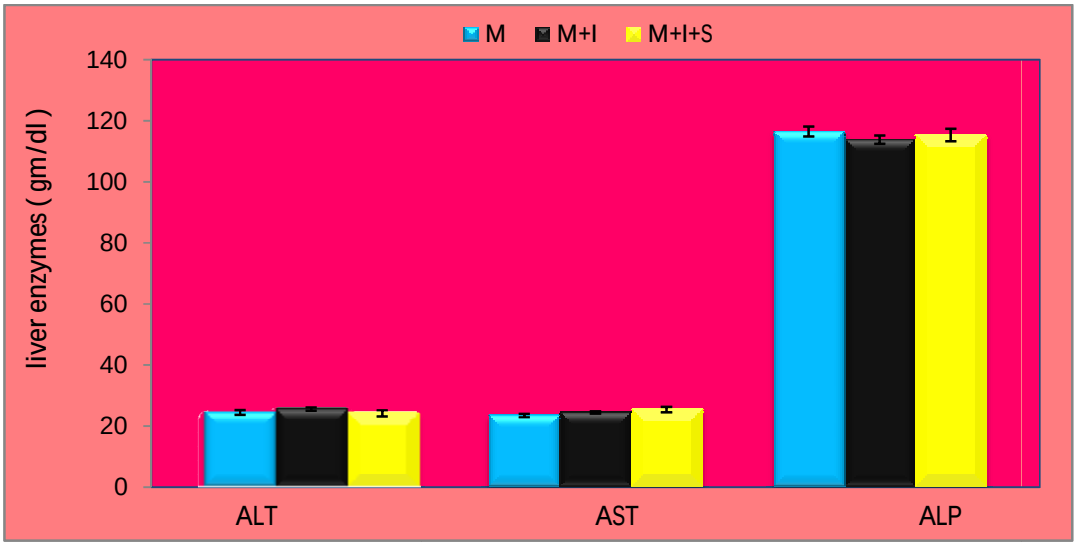
Table 10: Effect of metformin as monotherapy and in combination with insulin and simvastatin on Liver functions of type2 diabetic patients

	ALT(u/l)	AST(u/l)	ALP(u/l)	Bilrubin(mg/d)
Metformin	**24.5±0.7 p = 0.36**	**23.5±0.5 P = 0.11**	**116.5±1.6 P = 0.44**	**0.31±0.01**
Metformin+insulin	**25.6±0.5 p = 0.36**	**24.5±0.4 p=0.11**	**113.8±1.3 P = 0.44**	**0.33±0.09 P = 0.08**
Metformin+insulin+simvaststin	**24.2±1.0 p = 0.36**	**25.5±0.9 p=.11**	**115.4±2.0 P = 0.44**	****0.37±0.01 P = 0.006**

The data are expressed as mean ±SEM

****= highly significant increased as compared with the other treated groups.**

Fig 14: Effect of metformin as monotherapy and in combination with insulin and simvastatin on liver functions of type 2 diabetic patients.



Results expressed as mean ± SEM

M = metformin **I = insulin** **S = simvastatin**

ALT=Alanine amino transferase AST= Aspartate amino transferase

ALP = Alkaline phosphatase

No significant change between treated groups.

III.2.4. Effect of three drug regimens on renal functions of type 2 diabetic patients.

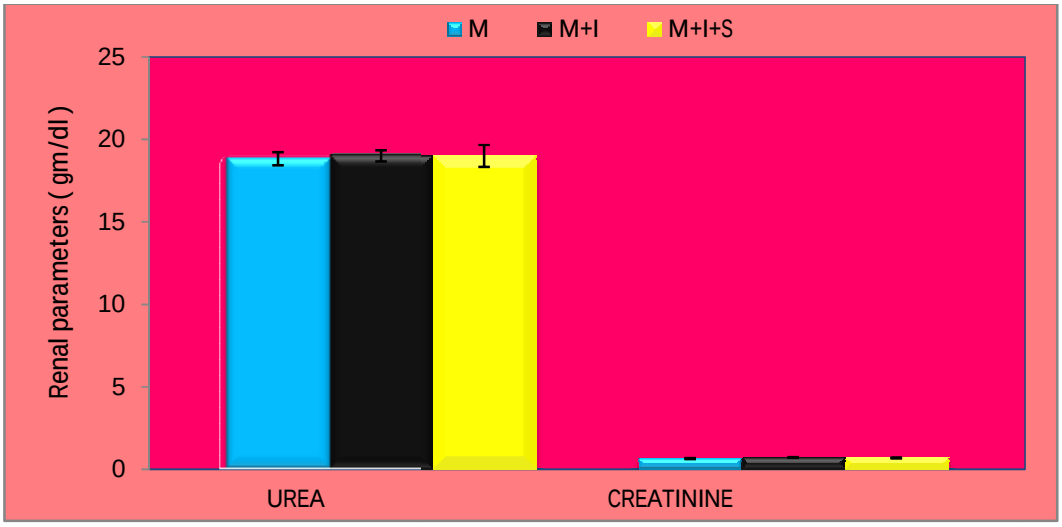
The statistic results and multiple comparisons among the three treated groups showed no significant change (P = 0.946) ,(P = 0.124), in urea and creatinine levels as presented in table(11) and fig(15).

Table 11: Effect of metformin as mono therapy and in combination with insulin and simvastatin on renal functions of type 2 diabetic patients

	Urea(mg/dl)	Creatinine(mg/dl)
Metformin	**18.8±0.39 P = 0.94**	**0.65±0.02 P = 0.12**
Metformin+insulin	**19.0±0.33 P = 0.94**	**0.71±0.02 P = 0.12**
Metformin+insulin+simvastastin	**19.0±0.66 P = 0.94**	**0.69±0.03 P = 0.12**

The data are expressed as mean ±SEM

Fig 15: Effect of metformin as monotherapy and in combination with insulin and simvastatin on renal functions of type 2 diabetic patients.



Results expressed as mean ± SEM

M = metformin **I = insulin** **S = simvastatin**

NO significant change between treated groups.

III. 3. Effect of different doses of simvastatin on lipid profile and AI of type 2 diabetic patients.

The statistical analysis study by one way ANOVA test followed by Post Hoc tests (LSD) data presented in the table(12) and fig (16), indicated that the level of LDL-C was very highly significant decreased ($P = 0.000$) at 40 mg simvastatin dose as compared to the lower doses 10mg and 20mg simvastatin. The data also revealed no significant reduction ($P < 0.52$) in the level of LDL-C at a dose of 20mg simvastatin as compared to 10mg dose. The level of TC was very highly significant decreased ($P = 0.000$), at a dose of 40mg simvastatin as compared to 10mg and 20mg dose, also there was no significant change ($P = 0.23$) in the level of TC at a dose of 20mg as compared to 10mg dose. The multiple comparisons of TG levels showed that there were no significant change ($P = 0.23$),between the three doses of simvastatin.

The statistical analysis also showed that the level of HDL-C at 40, 20,10 mg doses of simvastatin were not significantly changed,($P=0.61$).

The data presented in the table (12) and fig (17), showed that the value of AI was highly significant decreased($P < 0.005$, $P < 0.007$), in patients treated by 40mg simvastatin as compared to patients treated by 10mg and 20mg simvastatin. no significant change ($P = 0.53$), in value of AI at a dose of 20 as compared to 10 mg dose of simvastatin.

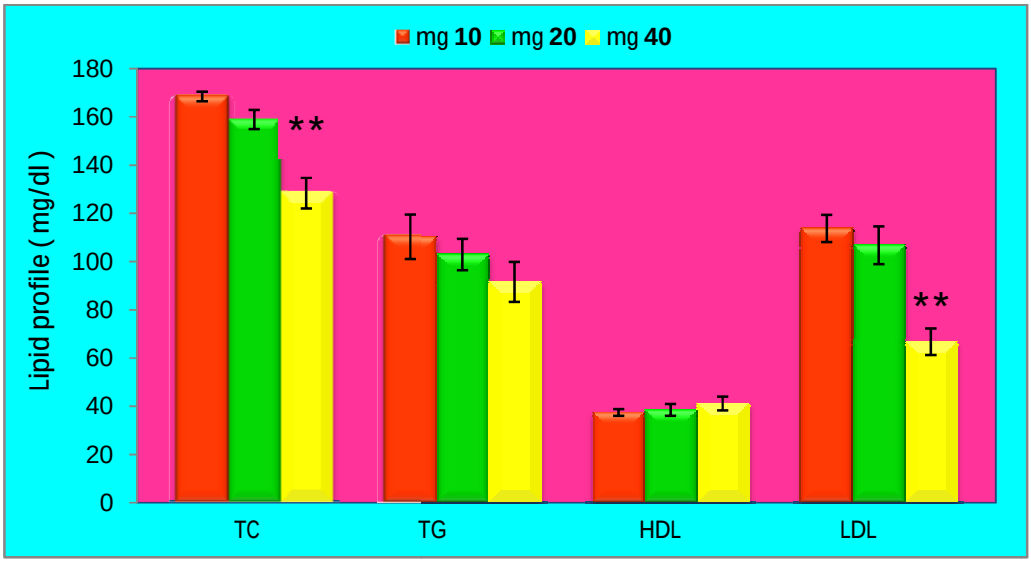
Table12: Serum lipid profile of Type 2 diabetic Patients taken various doses of simvastatin drug.

	TC(mg/dl)	TG(mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	AI=TC/HDL-C
Simvastatin (10mg)	168.5±1.9 P = 0.23	110.4±9.2 P = 0.29	37.5±1.3 P = 0.61	113.8±5.5 p = 0.52	4.6±0.13 P = 0.53
Simvastatin (20mg)	159.0±3.9 P = 0.23	103.0±6.5 P = 0.29	38.6±2.4 P = 0.61	106.9±7.8 P = 0.52	4.3±0.34 p=0.53
Simvastatin (40mg)	***128.5±6.3 P = 0.000	91.6±8.3 p= 0.29	41.2±2.8 p = 0.61	***66.9±5.5 P = 0.000	**3.2±0.25 p = 0.005 p = 0.007

The data are expressed as mean ±SEM

**** = Highly significant decreased when compared to groups treated with 10 mg and 20 mg simvastatin.**
***** = very Highly significant decreased when compared to groups treated with 10 mg and 20 mg simvastatin.**

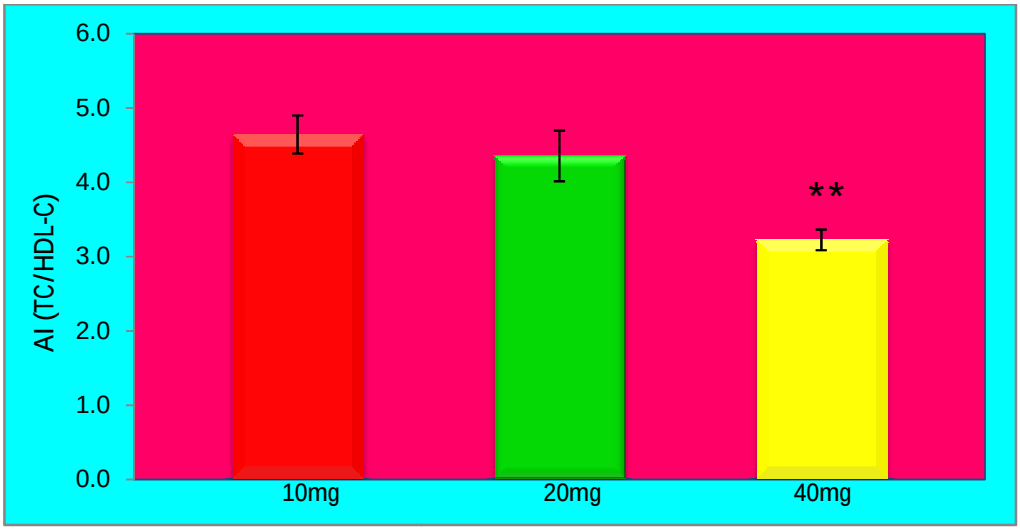
Fig 16: Effect of different doses of simvastatin on lipid profile of type2 diabetic patients



Results expressed as mean ± SEM

TC =Total cholesterol TG = Triglyceride HDL-C = High density lipoprotein
LDL-C = Low density lipoprotein
** highly significant decreased as compared to patients treated by 10mg & 20mg of simvastatin

Fig 17: Effect of different doses of simvastatin on AI of type 2 diabetic patients



Results expressed as mean ± SEM

** highly significant decreased as compared to patients treated by 10mg & 20mg of simvastatin

CHAPTER IV
DISCUSSION AND CONCLUSION

Metformin is the most common prescribed oral antidiabetic drug in the world It shall continue to maintain its position despite of several other classes of oral agents have been recently introduced both as initial therapy and in combination with these newer drugs for prevention and treatment of type 2 diabetes mellitus (DM).

The current study focuses on efficacy, safety profiles of metformin as a drug of first choice in the treatment of newly diagnosed patients with type 2 DM and to evaluate its efficacy and safety of prolonged treatment with metformin. Metformin is a hepato- selective insulin sensitizer it has beneficial properties including weight reduction, lipid reduction and as a modulator of endothelial function and an atherostatic agent, devoid of risk of hypoglycemia.

Metformin is effective as monotherapy and in combination with insulin and remains a safe and effective agent in treatment of type 2 DM. It is still in most circumstances the agent of choice for first line initial therapy of the typical obese patient with type 2 DM and mild to moderate hyperglycaemia. The most recent consensus statement for the management of type 2 diabetes from the ADA and the European Association for the Study of Diabetes (EASD) recommends metformin, due to its greater safety, as the best drug to be used together with lifestyle changes at the beginning of treatment (Nathan., al 2006).

The statistic in the study was done by paired data analysis, in which each patient was his own control, thereby increasing the power of the statistical analysis. Meformin is widely recognized to have either little effect on body weight or to facilitate modest weight loss in type 2 diabetes (Golay., 2008).

A meta-analysis of nine trials found an average difference in body weight of(-4Kg) (Campbll and Howlett.,1995). for type 2 diabetic patients treated by metformin and our reported results in agreement with this study and showed that metformin cause modest weight loss in diabetic patients and significant decreased in BMI in post treated as compared to pre treated diabetics.

The BMI (Queteite index) is the proxy for body fat percentage and according to WHO BMI =18.5-25 consider as optimal weight, BMI =30 is overweight, BMI < 30 is obese, overweight and obese individuals are at higher risk for many diseases like hypertension, dyslipidemia, type 2 DM, coronary heart disease, and there were several mechanisms have been postulated by which metformin might contribute to weight loss including,

- Reduction in GIT absorption of carbohydrates and decrease insulin resistance and hyperinsulinemia (Collier et al.,2006).
- Modulation of of Leptin (Glueck et al., 2001).
- Induction of a lipolitic and anoretic effect by acting on glucagon–like peptide (Mannucci., 2001).
- inhibition of DPP-4 which lead to delay of gastric empty and so suppression of appetite (Lindsay., 2005).
- Increasing thermogenic activity of brown adipose tissue,(Bailey, 1992)
- Redistribution of fat from visceral depots to subcutaneous depots (Adiposity), which is carry lesser cardiovascular risk (Kurukulasuriya et al.,1999).

Many previous reports showed that the weight loss was dose dependent, increasing with doses up and beyond the maximum of 2550mg/day of metformin (Grant .,1996) and this in

agreement with our result and we found that metformin at a dose of (2g/day), cause more significant weight reduction as compared with metformin at (1g/day) dose.

There is evidence that metformin having a favorable effect on blood sugar reduction ,and significant improvement on glycemic parameters. The effectiveness of metformin as an antidiabetic drug is explained by its ability to lower blood glucose by delaying intestinal absorption of glucose and decreasing gluconeogenesis in the liver, stimulating glucose uptake in the muscle, and increasing fatty acid oxidation in adipose tissue. The final effect is an improvement of peripheral insulin sensitivity (Stumvoll et al., 1995). According to the study by(Garber et al., 1997). the improvement in glucose profiles were dose dependent, he was found that metformin at a dose of 1g & 2g/day produce reduction on FBG by (-31mg/dl) and (-78mg/dl), respectively and HbA1c reduction by (-1.2 %) and (-2 %),respectively.

This is in agreement with our results since we found that ,therapy for 12 weeks with metformin cause significant improvement in all glycemic parameters, and showed that glucose-lowering efficacy of metformin in type 2 diabetes is dose related and showed nearly same reduction values in glycemic parameters with metformin at ,(1g&2g/day) in the treated patients.

The literatures show discrepant results about the influence of metformin on lipid profile (Wulffele et al., 2004)). Some studies reported reduction only in TC levels (Grant .,1996). while others reported reduction of TC and TG with an increase of HDL-C (Robinson et al., 1998 and Yki-Jarvinen et al., 1999). Still other studies showed no changes in lipid profile (Groop et al., 1989 and Rains et al., 1988)) . Another investigation showed an association of metformin with an improvement in the lipid profile even in non-diabetic patients (DeFronzo and Goodman., 1995).

Some studies in agreement with ours. Where we found that metformin improves lipid profile of diabetic patients and produce significant reduction in TC,TG, LDL-c levels and as well as produces a significant reduction in the AI value which is considered as predictive of ischemic heart disease risk and reflect the balance between the atherogenic and protective lipoprotiens of treated patients , but there was no significant change in the level of HDL-C , this findings in agreement with A meta-analysis of 41 randomized, controlled evaluations of metformin of at least 6- weeks duration showed significant reduction in TC, TG, LDL-C, levels in patients randomized to metformin relative to comparator treatments HDL-C was rarely improved by metformin (Wulffle et al ,2004). and as well as other previous study found a modest improvements in levels of TC, TG, LDL-C are often observed though little or no change in HDL-C level is usually seen after 12 weeks of metformin treatment (Nagi et al., 1993).

The present study also showed that the lipid lowering efficacy of metformin was significant at a dose of (2g/day), further more the authors reported that the effects of metformin on lipids showed dose-dependent effect , and TC and TG levels were decreased with high dose metformin (Grant .,1996).

Another study showed that the most effective dosage of metformin observed in the studies was (2g /day) (Garber et al., 1997) .

And the beneficial cardiovascular effect of metformin observed in the UK prospective diabetes study were obtained at median daily metformin dosage of 2500 mg/day (UK PDS.,

1998), this dose very closely to the dose at which the drug improves lipid profile. the reduction LDL-C associated with metformin therapy in our study is very important because of the LDL-C consider as atherogenic lipoproteins and have strong association with development of ischemic heart disease and the demonstration that a reduction in LDL-C is associated with a reduction of coronary mortality in non diabetic men (Shepherd et al., 1995). The expected mechanism by which metformin improve lipid profile is that metformin phosphorylates and activates AMP-activated protein kinase (AMPK) which affect the transcription of several key regulators of liver lipid production (lipogenesis) and hepatic gluconeogenesis (Zhou et al., 2001). The first regulation of lipogenesis is a reduction in the expression and activity of sterol regulatory element binding protein-1 (SREBP-1) which leads to two beneficial effects on lipids. One effect is a reduced expression of the enzyme fatty acid synthase, which leads to a reduction in fatty acid synthesis. These are both essential steps in the formation of triacylglycerols (also known as triglycerides (TG)) that make up the majority of VLDL being produced by the liver. Another effect is the phosphorylationof 3-hydroxy-3-methyl-glutary;-CoA reductase (HMG CoA Reductase) which reduces its cholesterol synthesis capabilities. The second regulation of lipogenesis is a phosphorylation of acetyl CoA carboxylase thereby inhibiting its activity. As a result, malonyl CoA levels are reduced leading to a reduction in fatty acid synthesis (need for TG production) and an enhancement of fatty acid oxidation.(Clarke et al.,1990).

Our study was also conducted to evaluated the effect of metformin on liver enzymes (ALT,AST,ALP) and bilirubin ,diabetic itself can causes change in the liver function test and cause an elevation in ALT and AST which is indicated risk for type 2 diabetes (Vozarova.,2002 and Hanley., 2004). However in this study, there was no change in the liver function tests was observed In the newly diagnosed type 2 diabetics except for about three patients showed elevation in ALT &AST levels due to certain diseases and they were excluded from our study. so this study focused on metformin change in liver function tests.

The results indicated that there was no significant difference in the level of hepatic transaminase and bilirubin in post treated as compare to pre treated patients and it has been shown by many studies that the level of hepatic transaminase and bilirubin were not changed after metformin treatment (Swisloki.,1998 .,2002and Islam.,2006).

Other study in contrast with our result it found that 4 weeks of metformin treatment produced an elevation in LFT and when metformin was discontinued LFT were normalized (Nammour et al., 2003). Also other study showed that the hepatotoxicity which rarely induced by metformin in type 2 diabetic patients was due to idiosyncratic adverse reaction to metformin or its metabolites (Deutsch et al., 2004).

In the present study there were no change in the renal function, (urea, creatinine) levels at the end of 12 weeks of metformin treatment. These results were consistent with (Salpeter and Greyber., 2010). they were showed that years of metformin use in type 2 diabetes patients, most patients did not have an elevated creatinine level and there are many studies demonstrated the role of metformin in the prevention of diabetic nephropathy through modulation of oxidative stress on the tubules and protection of renal tubular cell injury due to glycosuria there was no evidence or even a trend towards deterioration of renal or hepatic function with metformin ,new evidence suggests that metformin might actually

protect the kidneys and could help to preserve their function by increasing levels of AMPK (AMP kinase) (Gretchen., 2008).

Kim etal., 2012 suggested that diabetes induced podocyte loss in diabetic nephropathy could be suppressed by metformin through repression of oxidative injury. other study done by Rachmani., 2002 . in diabetic patients with elevated serum creatinine (2.4mg/dl), and continued metformin use there was no significant increased in serum creatinine or no cases of lactic acidosis over a 4-years period.

Since there were no significant change in the levels of hepatic trans-aminases , and no change in the levels of creatinine and urea which are good indicators of liver and kidney functions this revealed safety of metformin even at higher dose which is (2g/day).

The second part of study was a retrospective analysis of medical records of patients with type 2 diabetes undergoing treatment with metformin or combined therapy of metformin and insulin and the last group use metformin and insulin and simvastatin combination . The long term efficacy and safety of metformin was investigated and the results of comparison of different efficacy and safety variables as following.

In the present study we found that the levels FBG showed no significant difference among the treated groups and even through FBG fell very significantly glycemic control in metformin & insulin treated group but with no significant difference when compared metformin treated group, but all treated groups have a significant FBG levels control . Additionally the PPBG levels among the three treated groups showed good control, but the reduction in PPBG level was more significant in metformin& insulin treated group than metformin treated group.

Our study found that the HbA1c values of all treated groups within the target range. even metformin & insulin treated group showed better HbA1c control and these results were consistent with the evidence facts of synergistic effect of two anti hyperglycemic drugs combination (Wulffele et al., 2002). , and so the combination of metformin and insulin may be an attractive therapeutic option for patients with type 2 DM whose hyperglycemia is poorly controlled using insulin alone. Aviles et al.,1999 Stated that combination therapy with insulin & metformin cause more improvement in glycemic control and more reduce HbA1c in compare to increased frequency of dosage of insulin alone. Furthermore the results showed no significant difference in the levels FBG and PPBG, HbA1c in metformin + insulin treated group as compared to group treated by metformin+insulin+simvastatin, the HbA1c levels of patients treated by simvastatin was showed slightly elevation as compared to the other two groups, some reports showed that Statin use is associated with a rise of FPG in patients with and without diabetes (Sukhija et al., 2009). and others (Sattar et al., 2010). have identified a deterioration in glucose homoeostasis in patients treated with statins and this effect depends on Lipid solubility of statins, simvastatin has high lipid solubility and can enters extra hepatic cells easily and may suppress isoprenoid protein synthesis, consequently attenuating the action of insulin .But some studies suggest that the abnormality in FPG may translate into the clinical syndrome of diabetes with a rise in HbA1c .

The precise mechanisms by which statins effect glucose metabolism are unclear and the Suggested Mechanisms include:

- Statin therapy may exacerbate insulin resistance in vivo and invitro
- The data suggest that atorvastatin decreases adipocyte maturation and results in a decline in expression of GLUT 4 (glucose transporter), (Nakata et al., 2006).
- Statin therapy may reduce insulin secretion and the experimental data in rats have demonstrated that when pancreatic β- cells are incubated with statins, insulin secretion is reduced due to inhibition of glucose-stimulated calcium channels (Yada et al., 1999).
- Statins have the potential to alter glycemic control by decreasing various metabolites such as isoprenoid. Isoprenoid in particular enhances glucose uptake via GLUT- 4 in adipocytes (Chamerlia et al., 2001).
- Statin delayed ATP production in pancreatic beta cells and thereby impair insulin release (Mabuch et al., 2005).
- Simvastatin has been shown to inhibit glucose-induced increase in intracellular calcium in pancreatic beta cells leading to the inhibition of insulin secretion in a dose-dependent manner (Sasaki ., 2006).

Our study was also conducted to compare the effect of metformin as mono therapy with its effect when combined with insulin on lipid profile of type 2 diabetic patients and the results showed that there was no significant difference in the levels of TC, TG, LDL-C, HDL-C in metformin +insulin treated group as compare to metformin treated group and this effect may be due to the better glycemic control attained by both drugs regimen which enables additional improvement in lipid profile (Sona and Regi.,2009). Furthermore the results showed no significant difference in the AI values of two treated groups .

The results also showed that the levels TC, LDL-C were significantly decreased in metformin + insulin + simvastatin treated group as compared to patients treated by metformin+insulin and it is well established that Simvastatin act by inhibiting of HMG-COA- reductase the rate limiting step enzyme in cholesterol biosynthesis and so produce decrease TC, LDL-C and TG levels and slight increases in HDL-C level (Darioli et al.,1990).

Furthermore HDL-C level showed less significant increased in patients treated by simvastatin as compared to the second group while TG level showed no difference between the two groups. and due to the pervious effects of simvastatin on lipid parameters the AI value was significantly decreased in simvastatin treated group than the comparator group.

The result also showed that the simvastatin cause more significant decreased in TC&LDL-C levels and less significant increased in HDL-C level this result was in agreement with previous studies showed that simvastatin has more effect on T C &LDL-C levels than HDL-C level with little or no effect on TG level (Findlay M., 1989).

In the present study we found that there was no significant change in the levels ALT, AST, ALP and bilirubin in diabetic patients treated by metformin in compare with patients treated by metformin+insulin.

Lobevitz and Kreider., 2002. demonstrated that no evidence of hepatotoxic effect or ALT abnormality were observed in patient taking either metformin or insulin and no significant difference between patients and placebo and this was in agreement with our founding and

since the patients in two groups showed good glycemic control .This also supports the important link among glycemic control ,insulin resistance and hepatic function and suggests that improved glycemic control and improvement of insulin resistance can reduce mild chronic elevation of transamnitis often found in diabetic patients (Elizabeth and Harris ., 2005).

Furthermore our result showed that there was no significant change in ALT,AST and ALP levels in metformin+insulin treated group in compare to group treated by metformin + insulin+simvastatin, these results were in agreement with previous literatures showed that there were no significant change on LFT in simvastatin treated diabetic patients and no change in ALT&AST levels and demonstrated that there was no abnormality in biochemical safety tests and no consistent adverse clinical or biochemical effects were observed during three years therapy with simvastatin (Shepherd et al., 2002,Drioli ., 1990 and Scott ., 1991).

The American college of physicians suggest that type 2 diabetic patients with other CV risk factors should take statin for primary prevention of macro vascular complication. These patients do not need routine monitoring of LFT which on statins unless they have baseline abnormalities on LFT. or they are taking other drugs that could increase their risk of adverse events (Snow et al., 2004). And furthermore for diabetic patients with baseline transaminases less than three times the upper limit of normal, it's not contraindicated to initiate, continue on advance statins therapy as long as patients are carefully monitored. Only high dose statins therapy is associated with more frequent abnormalities of LFTS, although they are still relatively infrequent (Pasternak ., 2002 and Larosa ., 2005).

In contrast to bilirubin level which showed a significant increased in simvastatin treated patients furthermore the authors reported that the level of bilirubin increased after simvastatin treatment in dependent of changes in liver enzymes (Pernette ., 2011).

Also the assessment of renal function, (urea & ceratinine) showed no significant change in metformin treated patients as compare to metformin+insulin treated patients
As well as no significant change in urea and creatinine levels in metformin+insulin treated patients as compare to metformin+insulin+simvastatin treated patients.

Additionally the interesting point which was observed during the assessment of lipid profile of type 2 diabetic patients subjected to treatment by three different doses of simvastatin,(10mg, 20mg, 40mg), is that the dose dependent effect of simvastatin and by comparing the lipid modifying efficacy of simvastatin at the three different doses our statistical data showed no significant change in the levels of TC,TG, LDL-C in patients take 10mg as compared to patients taking 20mg simvastatin ,Although more significant decreased in TC, LDL-C levels were observed in patients treated 40mg as compared to patients treated by 10mg and 20mg of simvastatin respectively.

The HDL-C level showed slight but not significant improvement though out the three different doses.

Furthermore the AI showed a significant decreased at a dose of 40mg as compared the lower doses of simvastatin.

Similar results were reported by (Peter et al., 2003). that during comparing of lipid lowering efficacy of statins, simvastatin showed lesser efficacy in TC, LDL-C levels reducing and lesser percentage in HDL-C level increasing with simvastatin increased doses and across dose range, also our result in agreement with previous studies that showed that increasing the of simvastatin dose to 20mg/day resulted only in marginal further reduction of serum cholesterol concentrations (Arnadottir et al.,1994).

CONCLUSION

From this study we may conclude the following

1- Metformin improves clinical outcomes in type 2 diabetic patients by controlling glycemia,

2-Additional cardiovascular protective effects of metformin through the improvement of dyslipidemia ,and significant reduction on AI of the treated patients.

3-The efficacy of metformin in controlling glycemia and lipids in type 2 diabetic patients is dose dependent , generally requiring titration up to 2g/day or above to achieve optimal effect.

4- Metformin showed good safety profile on hepatic and renal functions of type 2 diabetic patients.

5-The efficacy of metformin in controlling hyperglycemia was enhanced when combined with insulin without negative effets.

6- Simvastatin as lipid lowering drug was very effective in controlling of dyslipidemia associated with diabetes and produce significant reduction in TC &LDL-C levels of treated patients.

7- The efficacy of simvastatin was dose dependent and showed maximum control at 40mg/day.

دراسة كلينيكة لمعرفة الفاعلية والآثار الجانبية لعقار الميتفورمين والأنسولين في علاج مرضي السكري من النوع الثاني

يعتبر عقار الميتفورمين من اكثر الادوية شيوعا في علاج مرض السكري خاصة مرضى النوع الثاني حديثي التشخيص ذوي الوزن الزائد . حيث اثبت فاعليته المتفورمين ليس فقط فى تنظيم سكر الدم وإنما ايضا فى تخفيض دهون الدم (الكولسترول و الدهون الثلاثية) المتلازمة عادة مع بداية داء السكرى وبتالى فله تأثير وقائى على القلب و الاوعية الدموية لمريض السكر وفى هذه الدراسة الشاملة لعقار المتفورمين التى اجريت على حوالى 60 مريض بداء السكر من النوع الثانى حديثى الاصابة لمعرفة فاعليته وتأثيراته الجانبية على وظائف الكبد و الكلى عند جرعتين مختلفتين 1 جم و 2 جم لدى مرضى السكر على مدى 12 أسبوع وقد اثبتت النتائج فاعلية عقار المتفورمين فى خفض سكر الدم عند استعماله 1جم و 2 جم بينما دلت نتائج الدراسة على ان المتفورمين عند الجرعة 1 جم لم يحدث اى تأثير على مستوي دهون الدم بينما عند الجرعة 2 جم احدث انخفضاً ملحوظاً في الدهون كما دلت النتائج ايضاً على ان المتفورمين لم يسبب اى تأثيرات جانبية على الكبد والكليتين حيث انه تحاليل وظائف الكلى مثل اليوريا و الكرياتينين وتحاليل وظائف الكبد مثل انزيمات الكبد و البيليروبين كانت ضمن الحدود الطبيعية لجميع افراد العينة المدروسة ودلت نتائج الجزاء الثاني من الدراسة والتي اجريت على 100 مريض من النوع الثاني ويتلقون نظام علاج معين مند اكثر من عامين وموزعون كالتى 30 مريض يعالجون بعقار الميتفورمين فقط و 40 مريض يعالجون بعقار الميتفورمين وعقار الانسولين والمجموعة الاخيرة 30 مريض يعالجون بعقار الميتفورمين والانسولين والسمفاستاتين.

على ان المرضى الذين يعالجون بعقار المتفورمين و الانسولين كان معدل السكر ضمن المعدل الطبيعى مع انخفاض اكثر فى معدل السكر التراكمى لديهم مقارنه بالمجموعة المعالجة بعقار المتفورمين فقط وأيضا تحاليل وظائف الكبد ووظائف الكلى كانت ضمن المعدل الطبيعى

كما اوضحت النتائج انه المرضى الذين يستخدمون المتفورمين والأنسولين و سمفاستاتين على انخفاض كبير فى مستوى دهون الدم مقارنة بالمجموعتين السابقتين.وكانت نتائج تحاليل وظائف الكبد والكلى ضمن المعدل الطبيعى فيما عدا ارتفاع ملحوظ في معدل البليروبين عند المرضى المعالجون بعقار السمفاستاتين (40 جم/اليوم). , ودلت النتائج انه عقار السمفاستاتين انتج معدل نقصان واضح فى مستويات الكوليسترول والدهون منخفضة الكثافة عند الجرعة (40 جم/اليوم) مقارنة بجرعات الاقل

(10جم - 20 جم/اليوم) وبالنسبة لمعدل الدهون الثلاثية والدهون عالية الكثافة فكانت ضمن الحدود الطبيعية مع عدم الاختلاف معدل النقصان او الزيادة لدى جميع الجرعات.

CHAPTER - V

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