# 1. Introduction and literature review

## 1.1. Diabetes Mellitus :

Is actually group of metabolic disorder characterized by hyperglycemia resulting from defect in the insulin secretion , insulin action or both.[2] Also it has been defined by the World Health Organization ( WHO ) , on the basis of laboratory findings as " a fasting venous plasma glucose concentration greater than 7.8 mmol\L (140 mg\dl) or greater than 11.1 mmol\L (200 mg\dl) two hour after a carbohydrate meal or two hour after the oral ingestion of the equivalent of 75g of glucose , even if the fasting concentration is normal".[1]

### 1.1.1. Maintenance of extracellular glucose :

Control of blood glucose is under two major hormones:

**I.Insulin**: is the most important hormones controlling plasma glucose concentration which it , in turn , controls ,Produce by β-cells of pancreatic islets .

Insulin binds to specific cell- surface receptors on adipose tissue and muscle and enhances the rate of glucose entry into these cells, The intracellular glucose concentration is kept low by insulin- induced activation of enzymes which stimulate its incorporation into glycogen synthesis (glycogenesis) in liver and muscle. Insulin also inhibits the production of glucose from fats and amino acids ( gluconeogenesis ) partly by reducing these substrates by inhibiting fat and proteins break down ( lipolysis and proteolysis ).

The transport of glucose into liver cells is insulin independent but , by reducing the intracellular glucose concentration , insulin does indirectly promote the passive diffusion of glucose into them . the normal response to hyperglycemia therefore depend on :

- Adequate insulin secretion .
- Normal insulin receptors .
- Normal intracellular reaction to receptor binding of insulin.[1]

**II.Glucagon:** is a single –chain polypeptide synthesized by the α-cells of pancreatic islets . Its secretion is stimulated by hypoglycemia . Glucagon enhances hepatic glycogenolysis and gluconeogenesis .When plasma insulin concentrations are low , for example during fasting , the hyperglycemic actions of other hormones , such as growth hormones ( GH ), glucocorticoids ,adrenaline and glucagon , become apparent , even if there is no increase in secretion rates.

Secretion of these hormones may increase during stress and in patients with acromegaly ( GH ) , cushing's syndrome ( glucocorticoids) or phaeochromocytoma ( adrenaline and nor adrenaline ) and thus oppose the normal action of insulin.[1]

1.1.2. **Classification of diabetes mellitus:**

Diabetes mellitus is classified into two major group:

•Primary diabetes mellitus.

•Secondary diabetes mellitus .

**A.primary diabetes mellitus :**

**1. Type 1 diabetes mellitus :**

It is term used to describe the condition in patients for whom insulin therapy is essential because they are prone to develop ketoacidosis . It usually present during childhood . It has been suggested that many cases fallow a viral infection , which has destroyed the β-cells of the pancreatic islets . Subject most at risk are those with HLA-types DR3 and DR4 of the major histocompatibility complex .[1]

Idiopathic type1 diabetes is form of type 1 that has no known etiology , is strongly inherited , and does not have β-cells autoimmunity . Individuals with this form of diabetes have episodic requirements for insulin replacement .[2]

**Causes of type1 DM :**

**I. Genetics :**

•Insulin autoantibodies.

•Islet cell autoantibodies .

•Glutamic acid decarboxylase autoantibodies .

Tyrosine phosphatase 1A-2 and 1A-2B autoantibodies [2]

**II.Environmental factors :**

usually virus in the individual with genetic predisposition .[2]

**2. Type 2 diabetes mellitus :**

Is commonest variety . Patients are much less likely to develop ketoacidosis than those with IDDM although insulin may sometimes be needed ,it is not essential for survival . Onset is most usual during adult life . Although no genetic markers have been found , there is familial tendency . A variety of inherited disorders may be responsible for the syndrome , either by reducing insulin deficiency because of resistance to its action or postreceptor defects ,despite high plasma insulin concentration .Factors increasing the risk of developing NIDDM include obesity ,sustained stress and a sedentary life style.[1]

•**Causes of type2 DM :**

Insulin resistance with insulin secretory defect and relative insulin deficiency.

**B. Secondary diabetes mellitus :**

-Absolute insulin deficiency , due to pancreatic disease ( chronic panceatitis , haemochromatosis ,cystic fibrosis )

-Relative insulin deficiency , due to excessive growth hormones ( acromegaly) , glucocorticoid secretion (cushing's syndrome ) , or increased plasma glucocorticoid concentration due to administration of steroids .

-Drugs such as thiazide diuretics .[1]

**C. Gestational diabetes mellitus (GDM) :**

Is any degree of glucose intolerance with onset or first recognition during pregnancy . Causes include metabolic and hormonal change ,patients with GDM frequently return to normal postpartum . However, this disease is associated with increased perinatal complications and increased risk for development of diabetes in later years. Infant born to mothers with diabetes are at increased risk for respiratory distress

syndrome , hypoglycemia , and hyperbilirubinemia .Fetal insulin secretion is stimulated in neonate of mother with diabetes.

### 1.1.3. **Pathophysiology of DM :**

In both type 1and 2 diabetes the individual will be hyperglycemic , which can be sever .glucosuria can also occur after the renal tubular transporter system for glucose becomes saturated . this is happens when the glucose concentration of the plasma exeeds rougly 180 mg/dl in an individual with normal renal function and urine output .as hepatic glucose overproduction continues ,the plasma glucose concentration reaches a plateau around 300-500 mg/dl.

The individual with type 1 diabetes has a higher tendency to produce ketones ,patient with type 2 diabetes seldom generate ketones , but instead have a greater tendency to develop hyperosmolar nonketotic states . The difference in glucagon and insulin concentration in these two groups appear to be responsible for the generation of ketones through increased β-oxidation. In type 1 , there is an absence of insulin with an excess of glucagon . This permits gluconeogensis and lipolysis to occur .in type 2 insulin is present as is (at time) hypeinsulinemia ; therefore, glucagon is attenuated, fatty acid oxidation is inhibited in type 2. This causes fatty acid to be incorporated into triglycerides as very-low density lipoproteins.

The laboratory findings of a patient with diabetes with ketoacidosis tend to reflect dehydration, electrolyte disturbances, and acidosis.

Acetoacetate, β- hydroxybutyrate , aceton are produced from the oxidation of fatty acids . The two former ketone bodies contribute to the acidosis. Lactate, fatty acid, and other organic acid can also contribute to a lesser degree. Bicarbonate and total carbon dioxide are usually decreased (deep respiration). This a compensatory mechanism to blow

off carbon dioxide and remove hydrogen ions in the process. The anion gap in this acidosis can exceed 16 mmol/L. serum osmolality is high as a result of hyperglycemia; sodium concentrations tent to be lower due in part to losses(polyuria) and in part to a shift of water from cells because of the hyperglycemia. The sodium value should not be falsey underestimated because of hypertriglceridemia.

More typical of untreated patient with type 2 diabetes is non ketotic hyperosmlar state . The individual presenting with this syndrome has an overproduction of glucose ; however, there appears to be an imbalance between production and elimination in urine . Often , this state is precipitated by heart disease , stroke , or pancreatits . Glucose concentration exceed300-500 mg/dl and sever dehydration is present .The sever dehydration contributes to inability to excrete glucose in urine. Mortality is high with this condition .Ketones are not observed because the sever hyperosmolar state inhibits the ability of glucagons to stimulate lipolysis . The laboratory findings of nonketotic hyperosmolar coma include plasma glucose value exceeding 1000mg /dl , normal or elevated plasma sodium or potassium , slightly decreased bicarbonate , elevated blood urea nitrogen (BUN) and creatinine , and elevated osmolality .(2

**1.1.4. complications :**

**A. acute complications :**
•**Diabetic ketoacidosis :**
Ketosis may  be the presenting feature of type 1 DM ,or may develp in apatient known to be diabetic who omits to take his insulin or whose insulin dosage becomes inadequate because of an increased requirement,for example as aresult of infection,any acute illness such as

myocardial infarction ,trauma or emotional disturbance.Newly diagnosed patients account for 20-25% of cases. The sequence of events which leads to hyperglycemia .Decreased peripheral utilization of glucose resulting from insulin lake and preferential metabolism of free fatty acids and ketone as energy substrate and increased secretions of catecholamines contribute to the hyperglycemia .

Insulin lake causes increased lipolysis, with increased release of free fatty acids into the blood from adipose tissue, and decreased lipogenesis .In the liver fatty acids normally undergo complete oxidation ,reesterified to triglycerides or are converted to acetoacetate and 3-hydroxybutyric acids.[3]

• **Lactic acidosis :**

Lactic acidosis is uncommon complication of diabetis. It was formerly chiefly seen in patients treated with phenformin, a bigunanide oral hypoglycemic drug ,but is now more usually associated with severe systemic illness ,for example severe shock and pancreatitis .[3]

• **Non ketotic hyperglycemia :**

Not all patients with uncontrolled develop ketoacidosis . In type 2 DM ,severe hyperglycemia can develop with extreme dehydration and a very high plasma osmolality ,but with no ketosis and minimal acidosis This complication is often refered to as hyperosmolar non ketotic hyperglycemia .[3]

B.**Late complications :**
1.**Macro vascular disease:**
The central pathological mechanism in macro vascular disease is the process of atherosclerosis, which leads to narrowing of arterial walls throughout the body. Atherosclerosis is thought to result from chronic inflammation and injury to the arterial wall in the peripheral or coronary vascular system. In response to endothelial injury and inflammation, oxidized lipids from LDL particles accumulate in the endothelial wall of arteries. Angiotensin II may promote the oxidation of such particles.

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Monocytes then infiltrates the arterial wall and differentiate into macrophages, which accumulate oxidized lipids to form foam cells. Once formed, foam cells stimulate macrophage proliferation and attraction of T-lymphocytes. T-lymphocytes, in turn, induce smooth muscle proliferation in the arterial walls and collagen accumulation. The net result of the process is the formation of a lipid-rich atherosclerotic lesion with a fibrous cap. Rupture of this lesion leads to acute vascular infarction..[4]

**2.Microvascular disease :**

The role of hyperglycemia in the development of microvascular complications of diabetes, such as nephropathy, retinopathy, and neuropathy, is well documented. The incidence of microvascular complications begins to increase at an HbA1c level >7.0% and increases by 30% to 40% per 1% increase in HbA1c level from over 8,000 patients . Microvascular complications are closely related to age, duration of diabetes, and glycemic control, and this relationship is stronger than that with macrovascular complications .[5]

**•Diabetic retinopathy:**

Diabetic retinopathy (DR) is a microvascular complication that can affect the peripheral retina, the macula, or both and is a leading cause of visual disability and blindness in people with diabetes.[6] The severity of DR ranges from nonproliferative and preproliferative to more severely proliferative DR, in which the abnormal growth of new vessels occurs.[7] Total or partial vision loss can occur through a vitreous hemorrhage or retinal detachment, and central vision loss can occur through retinal vessel leakage and subsequent macular edema.[8] The prevalence of DR increases with prolonged duration of diabetes.[9] In studies including people with both type 1 diabetes and type 2 diabetes, after 30 years of diabetes, most patients had some form of DR, and over half had proliferative DR; people with type 1 diabetes and taking insulin had the

highest prevalence of DR, and people with type 2 diabetes diagnosed after age 30 had the lowest prevalence of DR.[10-11]Diabetic retinopathy also recently was seen in approximately 10% of people with insulin resistance (prediabetes) and was associated with the presence of hypertension and a higher body mass index.[12]

**1.2.lipids and Lipoproteins:**

**1.2.1.Lipids :**

The lipids are a group of organic substances of fatty nature which are insoluble in water soluble in fat solvents , such as ether, alcohol, chloroform and benzene related to the fatty acids ( either actually or potentially )as esters and utilizable in metabolism by living organisms[13]

*Biological functions of lipids:**
1. They serve as the reservoir of high energy value.
2. They can be stored in concentrated form in water free state in the adipose tissue.
3. They are important components of cell membranes.
4. They form important constituent of nervous tissue.
5. They form insulating and protective coating in the subcutaneous tissues and around certain organs.
6. They are important dietary constituents in form of oil soluble vitamins and essential fatty acids.
7. Lipoproteins are important constituent of cell membrane and mitochondria.[13]

**1.2.2. Plasma lipid:**

**I.Fatty acids:**
Are straight –chain carbon compounds of varying lengths. They may be saturated, containing no double bonds, monounsaturated with one, or polyunsaturated with more than one , double bonds. Fatty acids may be esterified with glycerol to form triglycerides , or be nonesterified or free (NEFA or FFA). Plasma FFA liberated from adipose tissue are transported , mainly bound to albumin , to the liver and muscle where they are metabolized . They provide a significant proportion of the energy requirements of the body . [1]

## II.Triglycerides:

Are fatty acids esters of glycerol , each containing 3 different fatty acids.
[1] Each fatty acid in the triglyceride molecule can potentially be different in structure, thus producing many possible structural forms of triglycerides.[2] There are no charged groups or polar hydrophilic groups, making it very hydrophobic and virtually water insoluble. Because it has no charge, triglyceride is classified as a neutral lipid.[2]

**\*Absorption and metabolism :**

Because fats are water insoluble, special mechanisms are required to facilitate the intestinal absorption . During the process of digestion, pancreatic lipase, by cleaving off fatty acids, first converts dietary lipids into more polar compounds with amphipathic properties. Thus, triglycerides are transformed into monoglycerides and diglycerides . These amphipathic lipids in the intestinal lumen form large aggregates with bile acids called micelles. Lipid absorption occurs when the micelles come in contact with the microvillus membranes of the intestinal mucosal cells; Shortchain free fatty acids, with 10 or fewer carbon atoms, can readily pass directly into the portal circulation and are carried by albumin to the liver. The absorbed longchain fatty acids, monoglycerides, and diglycerides are reesterified in intestinal cells to form triglycerides then packaged into chylomicrons, along with apo B-48.[2]

They are transported from the intestine  and the liver to various tissues, such as adipose tissue, as lipoproteins. Plasma     triglycerides concentration rise after a fatty meal and remain increased for several hours.[1]

**\*Hormonal control:**

Triglycerides are influenced by a number of hormones, such as insulin, glucagon, pituitary growth hormone, adrenocorticotropic hormone (ACTH), thyrotropin, and adrenal medulla epinephrine an norepinephrine

from the nervous system. Epinephrine and norepinephrine influence serum triglyceride levels by triggering production of hormone-sensitive lipase, which is located in adipose tissue. Other body processes that trigger hormone sensitive lipase activity are cell growth (growt hormone), adrenal stimulation (ACTH), thyroid stimulation (thyrotropin), and fasting (glucagon). Each process, through its action on hormone-sensitive lipase, results in an increase in serum triglyceride values.[2]

### III. Phospholipids:

Are complex lipids, resembling triglyceride , but containing phosphate and a nitrogenous base in place of one of the fatty acids . They are important components of cell membranes and lipoproteins , maintaining the solubility of non –polar lipid and cholesterol.[1]

### IV. Cholesterol:

A steroid , is a precursor to many physiologically important steroieds ; such as bile acids and steroid hormones . Cholesterol synthesis initially involves the conversion of acetate to mevalonic acid .
About two –thirds of the plasma cholesterol is esterified with fatty acid to form cholesterol esters . unlike that of triglycerides plasma concentration of cholesterol dose not rise after a fatty meal.[1]

### 1.2.3.Lipoproteins:

As the name implies, lipoproteins are composed of both lipids and proteins, called apolipoproteins[2] . The core of insoluble cholesterol ester and triglycerides is surrounded by proteins , phospholipids and free cholesterol with their water-soluble groups facing outwards.[1]

### 1.2.3.1.Classification:

The various lipoprotein particles were originally separated by ultracentrifugation into different density fractions.[2]

### I.Chylomicrons:

Are the largest and the least dense of the lipoprotein particles . Because of their large size, they reflect light and account for the turbidity of postprandial plasma. Because they are so light, they also readily float to the top of stored plasma and form a creamy layer, which is a hallmark for the presence of chylomicrons . Chylomicrons are produced by the intestine, where they are packaged with absorbed dietary lipids. Once they enter the circulation, triglycerides and cholesteryl esters in chylomicrons are rapidly hydrolyzed by lipases and, within a few hours, they are transformed into chylomicron remnant particles, which are recognized by proteoglycans and remnant receptors in the liver, facilitating their uptake. The principal role of chylomicrons is the delivery of dietary lipids   to hepatic and peripheral cells.[2]

## II.    Very Low Density Lipoproteins( VLDL ):

VLDL is produced by the liver .like chylomicrons, they are also rich in triglycerides. They are the major carriers of endogenous (hepatic-derived) triglycerides and transfer triglycerides from the liver to peripheral tissue. Like chylomicrons, they also reflect light and account for most of

the turbidity observed in fasting hyperlipidemic plasma specimens, although they do not form a creamy top layer like chylomicrons, because they are smaller and less buoyant . Excess dietary intake of carbohydrate, saturated fatty acids, and trans fatty acids enhances the hepatic synthesis of triglycerides, which in turn increases VLDL production.[2]

## III.Low-Density Lipoproteins( LDL ):

They form as a consequence of the lipolysis of VLDL. LDL is readily taken up by cells via the LDL receptor in the liver and peripheral cells. In addition, because LDL particles are significantly smaller than VLDL particles and chylomicrons, they can infiltrate into the extracellular space

of the vessel wall, where they can be oxidized and taken up by macrophages through various scavenger receptors. Macrophages that take up too much lipid become filled with intracellular lipid drops and turn into foam cells, which is the predominant cell type of fatty streaks, an early precursor of atherosclerotic plaques.[2]

## IV.  High-Density Lipoproteins( HDL ):

The smallest and most dense lipoprotein particle, is synthesized by both the liver and intestine .[2]

*synthesis ,metabolism and action of HDL:

Esterification of cholesterol is catalysed by enzyme *lecitbin-cholesterol acyltransferase (LCAT) ,* which is part of the HDL particle and which is activated by apoA1.[1]

HDL can exist as either disk-shaped particles or, more commonly, spherical particles. Discoidal  HDL typically contains two molecules of apo A-I, which form a ring around a central lipid bilayer of phospholipid and cholesterol. Discoidal HDL is believed to represent nascent or newly secreted HDL and is the most active form in removing excess cholesterol from peripheral cells. The ability of HDL to remove cholesterol from cells, called reverse cholesterol transport, is one of the main mechanisms proposed to explain the antiatherogenic property of HDL. When discoidal HDL has acquired additional lipid, cholesteryl esters and triglycerides form a core region between its phospholipid bilayer, which transforms discoidal HDL into spherical HDL. HDL is highly heterogeneous separable into as many as 13 or 14 different subfractions. There are two major types of spherical HDL based on density differences: HDL2 and HDL3. HDL2 particles are larger in size and richer in lipid than HDL3 and may reflect better efficiency in delivering lipids to the liver.[2]

*HDL synthesis is increased by estrogen ; consequently plasma concentrations are higher in menstruating women than in women after the menopause or in men.[1]

### I.2.4. Disorders of plasma lipids:
### I. Hypertriglyceridemia:

Hypertriglyceridemia is a condition in which triglyceride levels are elevated, often caused or exacerbated by uncontrolled diabetes mellitus, obesity, and sedentary habits. This condition is a risk factor for coronary artery disease (CAD).[14]  Elevated levels may be due to an increase in plasma VLDL or chylomicrons or both.[1]

**\*Signs and symptoms:** Hypertriglyceridemia is usually asymptomatic until triglycerides are greater than 1000-2000 mg/dL. Signs and symptoms may include the following:

•GI: Pain in the mid-epigastric, chest, or back regions; nausea, vomiting.

•Respiratory: Dyspnea.

•Dermatologic: Xanthomas.

•Ophthalmologic: Corneal arcus, xanthelasmas.[14]

**\*Types:**

**a. Familial:** Hypertriglyceridemia consequence of genetic abnormalities. [2]

**b. Secondary:** to hormonal abnormalities associated with the pancreas, adrenal glands, and pituitary, or of diabetes mellitus or nephrosis.[2]

### II.    Hypercholesterolemia:

Is the lipid abnormality most closely linked to heart disease.[2]

  **\*causes:** The coexistence of an underlying genetic defect , or the development of a disorder that affects plasma LDL concentrations.[1]

**\* Types:**
**a. Primary :**   the familial incidence of it ,often associated with an increased risk of  ischaemic heart disease , suggests an inherited

disorder , the exact nature of which can only be established by extensive family studies.[1]

**b. Secondary:** to primary hypothyroidism , diabetes mellitus , nephritic syndrome , cholestasis and drugs.[1]

**III.** **Mixed Hyperlipidaemia:**

Raised plasma concentration of both cholesterol and triglycerides are commonest in patients with poorly controlled diabetes mellitus , severe hypothyroidism or the nephritic syndrome.[1]

### 1.2.5.Arteriosclerosis:

The relationship between heart disease and dyslipidemias stems from the deposition of lipids, mainly in the form of esterified cholesterol, in artery walls. This lipid deposition first results in fatty streaks, which are thin streaks of excess fat in macrophages in the subendothelial space. Fatty streaks can develop over time into plaques that contain increased number of smooth muscle cells, extracellular lipid, calcification, and fibrous tissue, which can partially block or occlude blood flow. Also, established plaque for unknown reasons can become vulnerable to rupture or erosion, triggering a thrombosis that can block circulation. when it develops in the heart, it is referred to as coronary artery disease (CAD). CAD is associated with angina and myocardial infarction..[2]

Plaque formation involves repeated cycles of cell injury, followed by infiltration and cell proliferation to repair the site. LDL is believed to play a central role in initiating and promoting plaque formation. It is deposited

into the subendothelial space where it is taken up by various cells, including macrophages. This alters the gene and protein expression pattern of these cells and can promote an inflammatory response, particularly when LDL becomes oxidized.

Injury signals from the evolving plaque trigger the expression of adhesion proteins on endothelial cells and the production of soluble chemotactic proteins from resident macrophages, which promotes the attachment and infiltration of additional macrophages, lymphocytes, and platelets to the plaque.

Continual injury and repair lead to additional narrowing of the vessel opening, or lumen, causing the blood to circulate in a nonlaminar manner under greater and greater pressure, which further aggravates plaque formation. The final event leading to complete occlusion of blood flow occurs when there is a hemorrhage into the plaque, which results in the formation of a thrombus that blocks blood flow and precipitates a myocardial infarction. Because lipid deposits in the vessel walls are frequently associated with increased serum concentrations of LDL cholesterol or decreased HDL cholesterol, lowering LDL is an important step in preventing and treating CHD.[2]

**1.2.6. Relation between Diabetes mellitus and lipid disorders:**

In type 2, insulin is present, as is (at times) hyperinsulinemia; therefore, glucagon is attenuated. Fatty acid oxidation is inhibited in type 2. This causes fatty acids to be incorporated into triglycerides for release as very low density lipoproteins (VLDL).[2]

Type 2 diabetes is associated with a marked increased risk of cardiovascular disease (CVD). Individuals with diabetes have an absolute risk of major coronary events similar to that of nondiabetic individuals with established coronary heart disease (CHD). Furthermore, after an acute coronary event, diabetic subjects develop congestive heart failure more frequently and have a higher mortality rate than nondiabetic individuals. A greater burden of risk factors is at least partly responsible for the increased risk of CHD in diabetes. Dyslipidemia is a

well-recognized and modifiable risk factor that should be identified early to institute aggressive cardiovascular preventive management.[15]

**1.3. Objectives:**

**1.3.1. General Objective:**

To determine the serum levels of triglycerides and high density lipoprotein cholesterol among Sudanese type 2 diabetic patients in references to BMI and gender.

**1.3.2. Specific Objectives**:

1. To measure serum levels of triglycerides and HDL-C in study groups .

2. To assess the serum levels of triglycerides and HDL-C in Sudanese type 2 diabetic patients according to their gender (males VS females).

3. To correlate the serum levels of triglycerides and HDL-C with the duration of diabetes mellitus.

4. To correlate the serum levels of triglycerides and HDL-C with BMI.

# 2. Materials and Methods

## 2.1. Materials:

### 2.1.1. Study design:

This is descriptive case control, hospital based study.

### 2.1.2. Study area:

The study was conducted in Advance Diagnostic Center in Khartoum state.

### 2.1.3 Study period:

The study was carried during the period from December 2013 to march 2014.

### 2.1.4. Study population:

The study was conducted on Sudanese type 2 diabetes mellitus (males and females) as test group and apparently healthy Sudanese individual (males and females) as control group.

Males and females were matched for age (30-70years).

### 2.1.5 Inclusion Criteria:

Test group: Sudanese type 2 diabetes mellitus (males and females).

### 2.1.6 Exclusion criteria:

Patients with type 1 diabetes mellitus , hypertension, hypothyroidism alcoholism, nephritic syndrome and those use certain medications (isotretinoin ) had been excluded.

### 2.1.7 Samples size:

Fifty Sudanese patients with type 2 diabetes mellitus males (25 males and 25 females) as a test group, and 20 apparently healthy Sudanese individual were enrolled in this study.

### 2.1.8. Ethical consideration:

All participate were told about the research importance during interview and all of them were accept to participate.

**2.1.9 Sample collection:**

After inform consent a local antiseptic (70% ethanol) was used to clean the skin. Venous blood (5ml) were taken from each participant by standard procedures, and placed in heparin anticoagulant and centrifuged at 3000 rpm for 3 minutes, then obtained plasma for triglycerides were separated in plain container and kept at -20C until used

**2.1.10 Equipments:**

- Auto analyzer.
- Centrifuge.
- Automatic pipette.
- Sterile needle.
- 70% alcohol
- Cotton.
- Constant temperature.
- Curettes.
- Test tubes.

**2.2 Methodology**

**2.2.1. Determination of triglyceride:**

 **Principle:**

The triglyceride  hydrolyzed into glycerol and fatty acid by lipase enzyme then glycerol phosphorylate  into glycerol-3-phosphate by glycerol kinase enzyme , glycerol-3-phosphate  oxidized by glycerol -3-phosphate oxidase into dihydroxyacetone phosphate and hydrogen peroxide which react with 4-amino phenazone  and phenol in the presence of peroxidase into Quinonimine ( pink colour) measure colorimetercally at 520nm .[12,13]


**Reagents:**
1. Reagent: Magnesium chloride 4.5mmol/L, phosphate buffer 50 mmol/L, 4-cholorophenol 5 mmol/L,lipoprotein  lipase$\geq$ 1.3 U/ml ,glycerol kinase $\geq$ 0.4 U/ml ,glycerol-3-phosphate oxidase

≥1.5 U/ml ,peroxidase 0.5 U/ml , 4-aminoantipyrine 0.25 mmol/L, ATP 2 mmol/L , PH 7.0.

2. Calibrator: it is recommended to use the Human multi-calibrator from Min dray and 9 g/L NaCl for two-point calibration. Traceability of multi-calibrator can refer to the calibrator instructions for use of Min dray Company.

**Reagent preparation:**

Reagent and Calibrator were ready to use.

**Technique:**

The reagents were first brought to room temperature, and then the following amounts were pipette according to the table below:

|  | Blank | Test |
|---|---|---|
| Working reagent | 1.0 ml | 1.0 ml |
| Distilled water | 10µL |  |
| Sample |  | 10µL |

Mixed thoroughly at 37Ċ, and read the absorbance 10 min, later at 520 nm.      ΔA=Abs sample –Abs blank

**Calculation:**

$$\frac{\text{Abs of Test} * \text{concentration of Standard} * \text{DF}}{\text{Abs of Standard}} = \text{concentration of Test}$$

**2.2.2. Determination of High Density Lipoprotein cholesterol:**

**Principle:**

1. Chylomicrons, Very Low Density Lipoproteins and Low Density Lipoproteins $\leftrightarrow$ Cholestenone +Hydrogen peroxide ($H_2O_2$).

In the presence of catalase $2H_2O_2 \leftrightarrow 2H_2O + O_2$

2. Cholesterol residue in High Density Lipoproteins hydrolyzed in presence of cholesterol esterase to free fatty acid and free cholesterol which oxidized by atmospheric oxygen in presence of cholesterol oxidase to cholestene-3 and hydrogen peroxide which converted by peroxidase to $H_2O$ and oxygen accepted by p-amino phenazone, in presence of HDAOS to produce Quinonimine (pink colour) the system monitor the change in absorbance at 600nm.

**Reagents:**

- Reagents: R1: cholesterol esterase 600 U/L, cholesterol oxidase 380 U/L, Good's buffer 100mmo/L, catalase 600KU/L, HDAOS 0.42mmol/L. R2: Good's buffer 100mmol/L , 4-aminoantipyrine 1 mmol/L ,peroxidase ≥ 2.8U/ml , surfactant ≤ 2 %
- Cholesterol Calibrator.

**Reagent preparations:**

Reagents and Calibrator were ready to use.

**Technique:**

|  | Blank | Test |
|---|---|---|
| R1 | 900µL | 900µL |
| Distilled water | 12µL |  |
| Sample |  | 12 µL |

Mixed, incubated for 5 minutes at 37Ċ, then added

| R2 | 300µL | 300µL |
|---|---|---|

Mixed thoroughly, incubated at 37Ċ for 5minutes, and then read the absorbance changed value.


**Calculation:**

Abs of Test X Concentration of Standard * D.F=Concentration of Test
          Abs Standard

**2.2.3. Quality control:**

The precision and accuracy of all methods used in this study were cheeked and were analyzed by commercially prepared control sera from Mind ray Company these control should be run with each new calibration, reagent cartridge and after specific maintenance or troubleshooting procedures.

## 2.2.4. Statistical analysis:

Data was analyzed by using the SPSS computer program. The means and standard deviation of serum level triglyceride and HDLc were detected and t-test was used for comparison (p.value of $< 0.05$ is considered to be significant).Linear regression analysis was used to assess correlation between the levels of triglyceride and HDLc and duration of diabetes mellitus and Body Mass Index.

# 3. Results

The study included 70 individuals , 50 of them with type 2 diabetes mellitus (25 males and 25 females) as test group and 20 were healthy individual as control group, were enrolled in this study. Test group and control group were matched for age (range 30-70 years).

**Serum Triglycerides**:

**Table (3.1):**

Show a insignificant difference between the means of serum triglyceride level of diabetic group compared to non diabetic group. (Mean ± SD): (mean=134±66) versus (118±38) mg/dL , respectively, ( P.value 0.17).

**Table (3.2):**

Show a insignificant difference between the means of serum triglyceride level of diabetic females group compared to the diabetic males group. (Mean ± SD): (137±75) versus (131±59) mg/dL, respectively, (p.value 0.728).

**Figure (3.1):**

A scatter plot shows insignificant very weak positive correlation between the BMI of diabetic group and the serum levels of triglyceride (r=0.058 P.value=0.690).

**Figure (3.2):**

A scatter plot shows significant weak positive correlation between the duration of diabetes mellitus and the serum levels of triglyceride and (r=0.355 P.value =0.011).

**Serum HDLc:**

**Table (3.1):**

Show a insignificant difference between the means of serum levels of HDLc of diabetic group compared to non diabetic group. (Mean ± SD): (45±12) versus (43±8), respectively, P.value (0.543) .

**Table (3.2):**

Show a significant difference between the means of serum levels of HDLc of diabetic females group compared to diabetic males. (Mean ± SD):  (48±19) versus (40±9), respectively, p.value (0.018) .

**Figure (3.3):**

A scatter plot shows insignificant very weak negative correlation between the BMI  of diabetic group and the serum levels of HDLc (r=-0.190 P.value 0.180).

**Figure (3.4):**

A  scatter plot shows insignificant very weak negative correlation between the duration of diabetes mellitus and the serum levels of HDLc , (r=-0.250 p.value=0.080 )

**Table (3-1): Comparison between the mean levels of serum Triglyceride and HDL in diabetic patients and non diabetic patients:**

| Variable | Diabetic patient N= 50 | Non    diabetic patient N =20 | p. value |
|----------|------------------------|-------------------------------|----------|
| Triglyceride | 134±66 | 112±38 | 0.170 |
| HDL | 45±12 | 43±8 | 0.543 |

-The table shows the mean ± Std deviation and probability (P.value ).

-T . test was used for comparison .

-P . value $\leq 0.05$ is considered significant .

**Table (3-2): Comparison between the mean levels of serum triglyceride and HDL in females and males diabetic patients:**

| variables | Diabetic females | Diabetic males | P .value |
|---|---|---|---|
| Triglyceride mg/dl | 137±75 | 131±59 | 0.728 |
| HDL mg/dl | 48±13 | 40±9 | 0.018 |

-The table shows the mean ± Std deviation and probability (P.value ) .

- T . test was used for comparison .

-P . value ≤ 0.05 is considered significant.
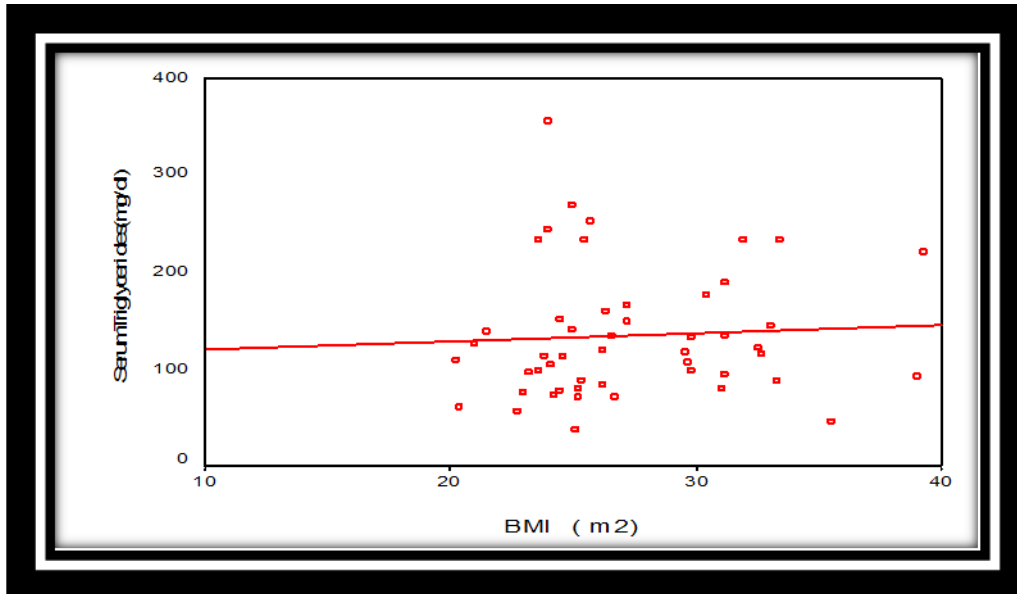
**Figure (3.1):**

A scatter plot shows correlation between the serum levels of triglyceride (mg/dl) and BMI ( $m^2$ ) of diabetic group.
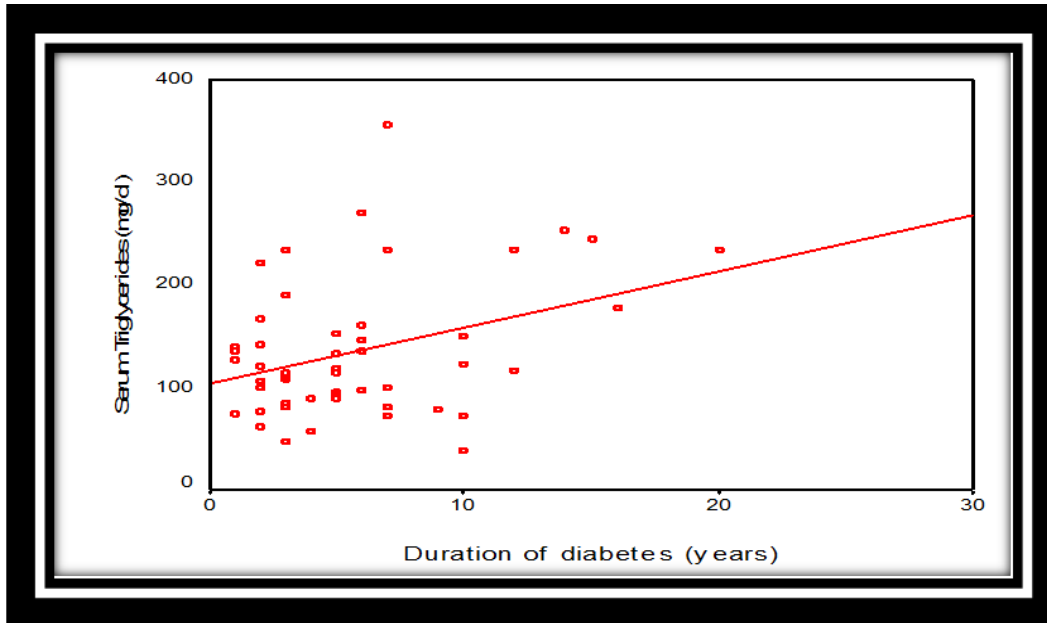
**Figure (3.2):**

A scatter plot shows correlation between the duration of diabetes mellitus (years) and serum levels of triglyceride(mg/dl) .
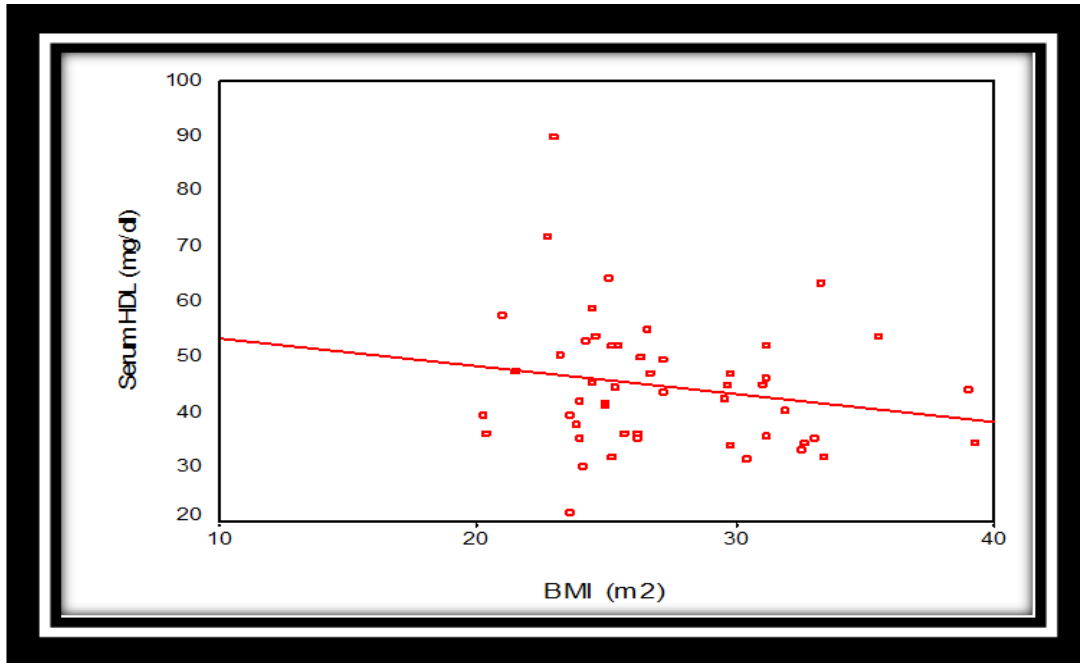
**Figure (3.3):**

A scatter plot shows correlation between the serum levels of HDLc (mg/dl) and BMI ( $m^2$ ) in diabetic group .
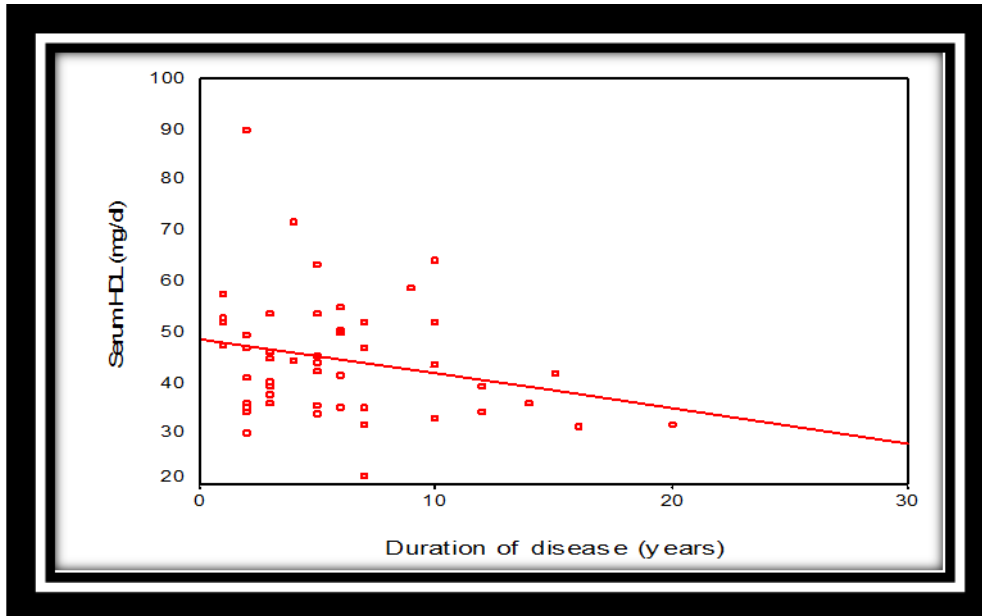
**Figure (3.4):**

A scatter plot shows correlation between the duration of diabetes mellitus (years) and serum levels of HDLc.

# 4. Discussion,Conclusion and Recommendations

## 4.1.Discussion :

Type 2 diabetes is associated with a marked increased risk of cardiovascular disease (CVD). Individuals with diabetes have an absolute risk of major coronary events similar to that of nondiabetic individuals with established coronary heart disease (CHD)[15].

In this study the serum levels of triglycerides and high density lipoprotein cholesterol were not significantly differ when compared with control group .Serum triglycerides levels was not significantly differ when diabetic males compared to diabetic females, while HDL-cholesterol levels was significantly differ between diabetic males compared to diabetic females. In study done by Walden C et al [16] ,who found that the serum levels of HDLc in diabetic females is less than diabetic males and the serum levels of triglyceride were not significantly differ between diabetic males and females.

In the diabetic group, there was significant positive correlation between the duration of diabetes mellitus and the serum levels of triglycerides, while showed an insignificant very weak correlation between the duration of diabetes mellitus and the level of HDL- Cholesterol. In study done by Talat N,et al [17], who found that duration of diabetes was associated with higher incidence of dislipidemia. In that study they found elevated triglycerides but normal HDL.

In the diabetic group, there were an insignificant very weak correlation between the BMI and the levels of serum triglycerides and HDL-Cholesterol. In the result done by Sandhu HS et al[18] ,who found that increase of BMI was associated with highest triglyceride level in addition to disturbance in other lipid profile. The result of this study

suggest that diabetic females may have increase risk to get cardiovascular disease (CVD) compared to diabetic males .

**4.2. Conclusion:**

The study concluded the following:

1. There was insignificant difference between serum levels of triglyceride and HDL of test group when compared with control group.
2. In reference to gender, the serum levels of triglyceride was insignificantly differ  between diabetic males compared diabetic females, whereas there was significant difference in the serum levels  of HDL between diabetic females compared to diabetic males.
3. There were significant positive  correlations between the duration of diabetes mellitus and serum levels of triglyceride, whereas there was insignificant negative correlations between the duration of diabetes mellitus and serum levels of HDL  . .
4. There was insignificant positive correlations between the BMI and the serum levels of triglyceride .
5. There were insignificant negative correlations between the BMI and the serum levels of  HDL .

## 4.3. Recommendations :

The study recommended the following:

1.Health education programs to improve people awareness about serious health effects of complications of diabetes.

2- Furthers studies should be done to measure the serum levels of triglyceride and HDL  in a large sample size .

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