1. Introduction

1.1 Background
Diabetes mellitus describes a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defect in insulin secretion or action or both. The effect of diabetes mellitus include long–term damage, dysfunction and failure of various organs diabetes mellitus may present with characteristic symptoms such as thirst, polyuria, blurring of vision and weight loss. In its most severe forms, Ketoacidosis, non-ketotic, death often symptoms are not severe, or may be absent consequently hyperglycemia sufficient to cause pathological and functional changes may be present for long time before the diagnosis is made. The long-term effects of diabetes mellitus include progressive development of specific complication of retinopathy with foot ulcers, amputation charcot joints, and features autonomic dysfunction, include sexual brovascular disease (kattenbash, et al., 2011).

Lipoproteins are spherical particles with a hydrophobic core (Triglyceride and estrified cholesterol). Lipoprotein analysis (lipid profile) measure Serum levels of total cholesterol, LDL cholesterol, HDL cholesterol, and Triglyceride. Plasma lipoproteins act as transporter for cholesterol and TG in the human blood (Salam, 2010). Diabetic patients have many complications which include elevated level of LDL-C and Triglyceride, low level of HDL-C and preponderance of abnormalities in the complication of smaller, dense particles (Smamth, et. al 2012).
2. Literature review

2.1 Diabetes mellitus:
Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels (Genuth et al., 2003).

2.1.1 Classification of Diabetes mellitus:
In 1979, the national diabetes data group developed a classification and diagnosis scheme for diabetes mellitus. This scheme included dividing into two broad categories type 1 insulin dependent diabetes mellitus (IDDM) and type II non-insulin dependent diabetes mellitus (NIDDM). Established in 1995, the international Expert committee on the diagnosis and classification of diabetes mellitus working under the sponsorship of the American Diabetes Association (ADA). Was given the task of updating the 1979 classification system the proposed changes included eliminating the older term of IDDM and NIDDM the categories of type 1 and type 2 were retained with adoption of Arabic numerals instead of Roman numerals. Therefor the ADA World health organization (WHO) guidelines recommend these categories which are type 1 diabetes, type 2 diabetes, other specific type of diabetes Gestational diabetes mellitus (Michael et al., 2010).

2.1.1.1 Type 1 Diabetes mellitus
Is characterized by appropriate hyperglycemia primarily results from pancreatic Islet β-cell destruction and tendency to ketoacidosis. Type2 diabetes in contrast includes hyperglycemia cases that results from insulin resistance with an insulin secretary defect an intermediate stage, in which the fasting glucose increase above normal limits but not to the level of diabetes, has been normal impaired fasting glucose .the term of impaired fasting glucose tolerance to indicate glucose tolerance values above normal but below diabetes level was retained. Also the term Gestational diabetes mellitus was retained for women who develop glucose intolerance during pregnancy. (Michael et al., 2010).

Type 1 diabetes mellitus is result of cellular –mediated autoimmune distraction of β-cell of pancreas, causing an absolute deficiency of insulin secretion. Upper limit 110mg/dl on fasting plasma glucose is designated as the upper limit of normal blood glucose. Type 1 constitutes only 10-20% of all diabetes and commonly occurs in childhood and adolescence. This disease is usually initiated by environmental factor or infection (usually virus). In individuals with genetic predisposition and causes the immune destruction of the β-cell of pancreas, and therefor a decreased production of insulin. Characteristic of type1 diabetes include abrupt onset, insulin
dependence and ketosis tendency. This diabetes is genetically related one or more of following markers are found in 85-90% of individual with fasting hyperglycemia islet cell auto antibodies, insulin autoantibodies, glutamic acid decarboxylase auto antibodies, and tyrosine phosphate IA2 and IA2B auto antibodies (Michael et al., 2010).

2.1.1.2 Type 2 diabetes mellitus
Type 2 DM is characterize by hyperglycemia as result of individual resistance to insulin secretary defect. this resistance results in relative, not an absolute, insulin deficiency, type2 constitutes the majority of the diabetes case, most patients in this type is obese or have increased percentage of body fat distribution in the abdominal region. This type of diabetes often goes undiagnosed for many years and is associated with strong genetic predisposition with patients at increase risk with age, obesity and lack of physical exercise. characteristics usually include adult onset of disease and milder symptom than type 1 with ketoacidosis seldom occurring. However these patients are more likely go into hyperosmolarcoma and are at increased risk of developing Macro vascular and micro vascular complication (Michael et al., 2010).

2.1.1.3 Other specific types of diabetes:
Genetic defects of the β-cell
Several forms of diabetes are associated with monogenetic defects in β-cell function. These forms of diabetes are frequently characterized by onset of hyperglycemia at an early age (generally before 25 years). They are referred to as maturity-onset diabetes of the young (MODY) and are characterized by impaired insulin secretion with minimal or no defects in insulin action. They are inherited in an autosomal dominant pattern. Abnormalities at six genetic loci on different chromosomes have been identified to date. The most common form is associated with mutations on chromosome 12 in a hepatic transcription factor referred to as hepatocyte nuclear factor (HNF)-1α. A second form is associated with mutations in the glucokinase gene on chromosome 7p and results in a defective glucokinase molecule. Glucokinase converts glucose to glucose-6-phosphate, the metabolism of which, in turn, stimulates insulin secretion by the β-cell. Thus, glucokinase serves as the “glucose sensor” for the β-cell. Because of defects in the glucokinase gene, increased plasma levels of glucose are necessary to elicit normal levels of insulin secretion. The less common forms result from mutations in other transcription factors, including HNF-4α, HNF-1β, insulin promoter factor (IPF)-1, and NeuroD1. Point mutations in mitochondrial DNA have been found to be associated with diabetes mellitus and deafness the most common mutation occurs at position 3243 in the t-RNA leucine gene, leading to an A-to-G transition. An identical lesion occurs in the MELAS syndrome (mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like syndrome); however, diabetes is not part of this syndrome, suggesting different phenotypic
expressions of this genetic lesion. Genetic abnormalities that result in the inability to convert proinsulin to insulin have been identified in a few families, and such traits are inherited in an autosomal dominant pattern. The resultant glucose intolerance is mild. Similarly, the production of mutant insulin molecules with resultant impaired receptor binding has also been identified in a few families and is associated with an autosomal inheritance and only mildly impaired or even normal glucose metabolism (Committee on the Diagnosis and Classification of Diabetes Mellitus 1997).

**Genetic defects in insulin action**

There are unusual causes of diabetes that result from genetically determined abnormalities of insulin action. The metabolic abnormalities associated with mutations of the insulin receptor may range from hyperinsulinemia and modest hyperglycemia to severe diabetes. Some individuals with these mutations may have acanthosis nigricans. Women may be virilized and have enlarged, cystic ovaries. In the past, this syndrome was termed type A insulin resistance. Leprechaunism and the Rabson-Mendenhall syndrome are two pediatric syndromes that have mutations in the insulin receptor gene with subsequent alterations in insulin receptor function and extreme insulin resistance. The former has characteristic facial features and is usually fatal in infancy, while the latter is associated with abnormalities of teeth and nails and pineal gland hyperplasia. Alterations in the structure and function of the insulin receptor cannot be demonstrated in patients with insulin-resistant lipoatrophic diabetes. Therefore, it is assumed that the lesion(s) must reside in the post receptor signal transduction pathways (Diabetes Care 2009).

**Diseases of the exocrine pancreas**

Any process that diffusely injures the pancreas can cause diabetes. Acquired processes include pancreatitis, trauma, infection, pancreatectomy, and pancreatic carcinoma. With the exception of that caused by cancer, damage to the pancreas must be extensive for diabetes to occur; adrenocarcinomas that involve only a small portion of the pancreas have been associated with diabetes. This implies a mechanism other than simple reduction in β-cell mass. If extensive enough, cystic fibrosis and hemochromatosis will also damage β-cells and impair insulin secretion. Fibrocalculouspancreatopathy may be accompanied by abdominal pain radiating to the back and pancreatic calcifications identified on X-ray examination. Pancreatic fibrosis and calcium stones in the exocrine ducts have been found at autopsy. Endocrinopathies several hormones (e.g., growth hormone, cortisol, glucagon, epinephrine) antagonize insulin action. Excess amounts of these hormones (e.g., Acromegaly, Cushing's syndrome, glucagonoma, pheochromocytoma, respectively) can cause diabetes. This generally occurs in individuals with preexisting defects in insulin secretion, and hyperglycemia typically resolves when the hormone excess is resolved. Somatostatinoma and aldosteronoma-induced hypokalemia can cause diabetes, at least in

**Drug- or chemical-induced diabetes**

Many drugs can impair insulin secretion. These drugs may not cause diabetes by themselves, but they may precipitate diabetes in individuals with insulin resistance. In such cases, the classification is unclear because the sequence or relative importance of β-cell dysfunction and insulin resistance is unknown. Certain toxins such as Vacor (a rat poison) and intravenous pentamidine can permanently destroy pancreatic β-cells. Such drug reactions fortunately are rare. There are also many drugs and hormones that can impair insulin action. Examples include nicotinic acid and glucocorticoids. Patients receiving α-interferon have been reported to develop diabetes associated with islet cell antibodies and, in certain instances, severe insulin deficiency. (Diabetes Care 2009)

### 2.2.1.4 Gestational diabetes mellitus (GDM): GDM is defined as any degree of glucose intolerance with onset or first recognition during pregnancy. The definition applies regardless of whether insulin or only diet modification is used for treatment or whether the condition persists after pregnancy. It does not exclude the possibility that unrecognized glucose intolerance may have antedated or begun concomitantly with the pregnancy. GDM complicates ~4% of all pregnancies in the U.S., resulting in ~135,000 cases annually. The prevalence may range from 1 to 14% of pregnancies, depending on the population studied. GDM represents nearly 90% of all pregnancies complicated by diabetes. Deterioration of glucose tolerance occurs normally during pregnancy, particularly in the 3rd trimester (Carpenter and Coustan 1982).

### 2-1-2 Diabetes Symptoms:

Common symptoms of both type 1 and type 2 diabetes include:

- Fatigue, constantly tired: In diabetes, the body is inefficient and sometimes unable to use glucose for fuel. The body switches over to metabolizing fat, partially or completely, as a fuel source. This process requires the body to use more energy. The end result is feeling fatigued or constantly tired. Unexplained weight loss: People with diabetes are unable to process many of the calories in the foods they eat. Thus, they may lose weight even though they eat an apparently appropriate or even an excessive amount of food. Losing sugar and water in the urine and the accompanying dehydration also contributes to weight loss. Excessive thirst (polydipsia): A person with diabetes develops high blood sugar levels, which overwhelms the kidney's ability to reabsorb the sugar as the blood is filtered to make urine. Excessive urine is made as the kidney spills the excess sugar. The body tries to counteract this by sending a signal to the brain to dilute the blood, which translates into
thirst. The body encourages more water consumption to dilute the high blood sugar back to normal levels and to compensate for the water lost by excessive urination.

- Excessive urination (polyuria): Another way the body tries to rid the body of the extra sugar in the blood is to excrete it in the urine. This can also lead to dehydration because a large amount of water is necessary to excrete the sugar.

- Excessive eating (polyphagia): If the body is able, it will secrete more insulin in order to try to manage the excessive blood sugar levels. Moreover, the body is resistant to the action of insulin in type 2 diabetes. One of the functions of insulin is to stimulate hunger. Therefore, higher insulin levels lead to increased hunger. Despite increased caloric intake, the person may gain very little weight and may even lose weight.

- Poor wound healing: High blood sugar levels prevent white blood cells, which are important in defending the body against bacteria and also in cleaning up dead tissue and cells, from functioning normally. When these cells do not function properly, wounds take much longer to heal and become infected more frequently. Long-standing diabetes also is associated with thickening of blood vessels, which prevents good circulation, including the delivery of enough oxygen and other nutrients to body tissues.

- Infections: Certain infections, such as frequent yeast infections of the genitals, skin infections, and frequent urinary tract infections, may result from suppression of the immune system by diabetes and by the presence of glucose in the tissues, which allow bacteria to grow. These infections can also be an indicator of poor blood sugar control in a person known to have diabetes.

- Altered mental status: Agitation, unexplained irritability, inattention, extreme lethargy, or confusion can all be signs of very high blood sugar, ketoacidosis, hyperosmolar hyperglycemia non-ketotic syndrome, or hypoglycemia (low sugar). Thus, any of these merit the immediate attention of a medical professional.

- Blurry vision: Blurry vision is not specific for diabetes but is frequently present with high blood sugar levels (E medicine health 2014).

2.1.3 Complications of diabetes:
People with diabetes have an increased risk of developing a number of serious health problems. Consistently high blood glucose levels can lead to serious diseases affecting the heart and blood vessels, eyes, kidneys, nerves and teeth. In addition, people with diabetes also have a higher risk of developing infections. In almost all high-income countries, diabetes is a leading cause of cardiovascular disease, blindness, kidney failure, and lower limb amputation. Maintaining blood
glucose levels, blood pressure, and cholesterol at or close to normal can help delay or prevent diabetes complications. Therefore people with diabetes need regular monitoring.

Cardiovascular disease: affects the heart and blood vessels and may cause fatal complications such as coronary artery disease (leading to heart attack) and stroke. Cardiovascular disease is the most common cause of death in people with diabetes. High blood pressure, high cholesterol, high blood glucose and other risk factors contribute to increasing the risk of cardiovascular complications.

Kidney disease (diabetic nephropathy): caused by damage to small blood vessels in the kidneys leading to the kidneys becoming less efficient or to fail altogether. Kidney disease is much more common in people with diabetes than in those without diabetes. Maintaining near normal levels of blood glucose and blood pressure can greatly reduce the risk of kidney disease.

Nerve disease (diabetic neuropathy): diabetes can cause damage to the nerves throughout the body when blood glucose and blood pressure are too high. This can lead to problems with digestion, erectile dysfunction, and many other functions. Among the most commonly affected areas are the extremities, in particular the feet. Nerve damage in these areas is called peripheral neuropathy, and can lead to pain, tingling, and loss of feeling. Loss of feeling is particularly important because it can allow injuries to go unnoticed, leading to serious infections and possible amputations. People with diabetes carry a risk of amputation that may be more than 25 times greater than that of people without diabetes. However, with comprehensive management, a large proportion of amputations related to diabetes can be prevented. Even when amputation takes place, the remaining leg and the person’s life can be saved by good follow-up care from a multidisciplinary foot team. People with diabetes should regularly examine their feet.

Eye disease (diabetic retinopathy): most people with diabetes will develop some form of eye disease (retinopathy) causing reduced vision or blindness. Consistently high levels of blood glucose, together with high blood pressure and high cholesterol, are the main causes of retinopathy. It can be managed through regular eye checks and keeping glucose and lipid levels at or close to normal.

Pregnancy complications: Women with any type of diabetes during pregnancy risk a number of complications if they do not carefully monitor and manage their condition. To prevent possible organ damage to the fetus, women with type 1 diabetes or type 2 diabetes should achieve target glucose levels before conception. All women with diabetes during pregnancy, type 1, type 2 or gestational should strive for target blood glucose levels throughout to minimize complications. High blood glucose during pregnancy can lead to the fetus putting on excess weight. This can lead to problems in delivery, trauma to the child and mother, and a sudden drop in blood glucose for the child after birth (International Diabetes Federation 2014).
2.1.4 Pathophysiology of Diabetes

An understanding of the Pathophysiology of diabetes rests upon knowledge of the basics of carbohydrate metabolism and insulin action. Following the consumption of food, carbohydrates are broken down into glucose molecules in the gut. Glucose is absorbed into the bloodstream elevating blood glucose levels. This rise in glycemia stimulates the secretion of insulin from the beta cells of the pancreas. Insulin is needed by most cells to allow glucose entry. Insulin binds to specific cellular receptors and facilitates entry of glucose into the cell, which uses the glucose for energy. The increased insulin secretion from the pancreas and the subsequent cellular utilization of glucose results in lowered blood glucose levels. Lower glucose levels then result in decreased insulin secretion. If insulin production and secretion are altered by disease, blood glucose dynamics will also change. If insulin production is decreased, glucose entry into cells will be inhibited, resulting in hyperglycemia. The same effect will be seen if insulin is secreted from the pancreas but is not used properly by target cells. If insulin secretion is increased, blood glucose levels may become very low (hypoglycemia) as large amounts of glucose enter tissue cells and little remains in the bloodstream. Following meals, the amount of glucose available from carbohydrate breakdown often exceeds the cellular need for glucose. Excess glucose is stored in the liver in the form of glycogen, which serves as a ready reservoir for future use. When energy is required, glycogen stores in the liver are converted into glucose via glycogenolysis, elevating blood glucose levels and providing the needed cellular energy source. The liver also produces glucose from fat (fatty acids) and proteins (amino acids) through the process of gluconeogenesis. Glycogenolysis and gluconeogenesis both serve to increase blood glucose levels. Thus, glycemia is controlled by a complex interaction between the gastrointestinal tract, the pancreas, and the liver. Multiple hormones may affect glycemia. Insulin is the only hormone that lowers blood glucose levels. The counter-regulatory hormones such as glucagon, catecholamines, growth hormone, thyroid hormone, and glucocorticoids all act to increase blood glucose levels, in addition to their other effects. Following meals, the amount of glucose available from carbohydrate breakdown often exceeds the cellular need for glucose. Excess glucose is stored in the liver in the form of glycogen, which serves as a ready reservoir for future use. When energy is required, glycogen stores in the liver are converted into glucose via glycogenolysis, elevating blood glucose levels and providing the needed cellular energy source. The liver also produces glucose from fat (fatty acids) and proteins (amino acids) through the process of gluconeogenesis. Glycogenolysis and gluconeogenesis both serve to increase blood glucose levels. Thus, glycemia is controlled by a complex interaction between the gastrointestinal tract, the pancreas, and the liver. Multiple hormones may affect glycemia. Insulin is the only hormone that lowers blood glucose levels. The
counter-regulatory hormones such as glucagon, catecholamines, growth hormone, thyroid hormone, and glucocorticoids all act to increase blood glucose levels, in addition to their other effects (Rumenian medical network 2006).

2.2 Lipid profile
Lipids are fat substances that provide energy to the body; are necessary for the production of steroid hormones and bile acids; and have a role in creating cell membranes. Two dominant lipids are cholesterol and triglyceride. Cholesterol and triglycerides are transported in the bloodstream by lipoproteins, which are complex molecules consisting of plasma proteins and lipids. Lipoproteins are categorized as high-density lipoproteins (HDL), cholesterol-rich plasma proteins; very-low-density lipoproteins (VLDL), triglyceride-rich plasma proteins; and low-density lipoproteins (LDL), the cholesterol-rich product of very-low-density lipoprotein breakdown, a lipid profile includes measuring plasma levels of cholesterol, triglycerides, HDLs, LDLs, and VLDLs. The purpose of the lipid profile is to detect disorders of lipid metabolism and to assess the risk of atherosclerosis, arteriosclerotic heart disease (ASHD), and peripheral vascular disease (Moisio and Moisio 1998).

2.2.1 Cholesterol
Cholesterol is a waxy substance that comes from two sources: the body and food. The body, and especially the liver, makes all the cholesterol body needs and circulates it through the blood. But cholesterol is also found in foods from animal sources, such as meat, poultry and full-fat dairy products. the liver produces more cholesterol when you eat a diet high in saturated and trans fats (American Heart Association 2014).

2.2.1.1 Functions of cholesterol
   a) It is important component of the membrane of cells.
   b) It is the major precursor for the synthesis of vitamin D.
   c) It is the major precursor of the steroid hormones, including cortisol, cortisone, and aldosterone in the adrenal glands.
   d) It is the major precursor of the sex hormone; progesterone, estrogen, and testosterone.
   e) Cholesterol molecule has an important role for the brain synapses as well as in the immune system (Salam, 2010).

Normal range
Total cholesterol: Less than 200 mg/dl
Border line: 200-240 mg/dl
High risky: > 240 mg/dl (Salam, 2010).
2.2.1.3 Causes of hypercholesterolemia
Include Arteriosclerosis, Nephritic syndrome, familial hyperlipidemia, coronary artery disease (CAD), high intake, pancreatitis, hypothyroidism and early stage of hepatitis (Salam, 2010).

2.2.1.4 Causes of hypocholesterolemia
Include Mal-nutrition, mal-absorption, hyperthyroidism, liver disease, pernicious anemia and sepsis (Salam, 2010).

2.2.1.5 Methods of estimation:
Chemical method which include:

- Libermann-Burchard method: this method measured the cholesterol extracted into cold chloroform and then treated with acetic anhydride, acetic acid, and concentrated sulphuric acid to form a green color complex.
- Zak’s ferric chloride method.
- Henly method (Salam 2010)

Enzymatic method:
Principle:
Cholesterol esters are hydrolyzed by cholesterol esterase enzyme to free cholesterol and free fatty acids. Free cholesterol is oxidized by cholesterol oxidize enzyme to form cholesta-4-ene-3-one and hydrogen peroxide. The hydrogen peroxide is reduced by hydrogen peroxidase enzyme to water and oxygen that is received by oxygen receptor (4-amino antipyrine), and in the presence of phenol as indicator-quinonimine red is formed and it is measured at 515nm green filter (Salam 2010).

2.2.2 Triglycerides
Triglycerides are formed from a single molecule of glycerol, combined with three fatty acids on each of the OH groups, and make up most of fats digested by humans. Ester bonds form between each fatty acid and the glycerol molecule. This is where the enzyme pancreatic lipase acts, hydrolyzing the bond and ‘releasing’ the fatty acid. In triglyceride form, lipids cannot be absorbed by the duodenum. Fatty acids, monoglycerides (one glycerol, one fatty acid) and some diglycerides are absorbed by the duodenum, once the triglycerides have been broken down (Brown and Goldstein 1997).

Normal range:
Normal: <150 mg/dl
Border line: 150-199mg/dl
High: 200-499 mg/dl
Very high: >500 mg/dl (Salam, 2010).
2.2.2.2 Causes of high result
a) Genetic: about 0.2% of hypertriglyceridemic patients are due to genetic affect.
b) Acquired: such as; DM, obesity, high level of lipoprotein, alcohol abuse, Hypothyroidism, nephrotic syndrome, hyperparathyroidism and renal failure (Salam, 2010).

2.2.2.3 Causes of low result
Mal-nutrition, mal-absorption and some drugs (Salam, 2010).

2.2.2.4 Methods of estimation
Measurement of serum triglycerides in conjunction with cholesterol is useful in detecting certain genetic and other types of metabolic disorders, as well as in characterizing risk of CVDs. The triglyceride value is also commonly used in the estimation of LDL cholesterol by the Friedewald equation. Chemical methods of estimation of TG were long, hazardous, and tedious procedures. So the enzymatic method represent greater cost reagents saving the technologist time and hazards (Sethi et al., 2010).

Enzymatic method
Principle:
TGs are broken down by lipase enzyme to glycerol and free fatty acids. And in the presence of ATP, the glycerol is phosphorilated by glycerol kinase enzyme to glycerol-3-phosphate. Then, the reaction can be completed by one of the following methods:
1. glycerol-3-phosphate reduces NAD+ catalyzed by glucose-6-phosphate dehydrogenase enzyme to give dihydroxyacetone phosphate (DHAP), hydrogen ions and NADH that read at 340nm.
2. glycerol-3-phosphate by an enzyme that called L-glycerophosphate oxidase enzyme gives dihydroxyacetone phosphate (DHAP) and hydrogen peroxide. The hydrogen peroxide is reduced by hydrogen peroxidase enzyme to water and oxygen that is received by oxygen receptor (4-amino antipyrine), and in the presence of phenol as indicator- quinonimine red is formed and it is measured at 515nm green filter (Salam, 2010).

2-2-3 Low Density Lipoprotein (LDL)
The breakdown of VLDLs is a major source of low-density lipoproteins (LDLs), which are cholesterol-rich plasma proteins. Increased levels of very-low density lipoprotein are accompanied by increased levels of low-density lipoproteins.
Low-density lipoprotein, a primary transporter of cholesterol, delivers and deposits the cholesterol into the peripheral tissues. Because of this function, LDLs are sometimes referred to as "bad" cholesterol and are associated with atherosclerosis, ASHD, and peripheral vascular disease (Moisio and Moisio, 1998).
2.2.3.1 Measurement of LDL-cholesterol
Several methods have been used to measure LDL-C. The first reference laboratory procedure, involve ultracentrifugation to separate LDL from other lipoproteins, followed by analysis as cholesterol. Finally, more recently developed homogeneous methods for measuring LDL-C are now used. A much more common second method uses the Friedewald formula to calculate LDL-C (Salam 2010).
Friedewald’s equation: \( \text{LDL} = \text{Total cholesterol} - (\text{HDL} + \text{VLDL}) \)

2.3.4 High Density Lipoprotein (HDL):
High-density lipoproteins (HDL) are plasma proteins that function as carriers of plasma cholesterol. Measuring the cholesterol contained in the HDL molecule is predictive of the individual’s risk for coronary artery disease. It is believed that the HDL molecule carries cholesterol from the peripheral tissues of the body to the liver, where the cholesterol is converted into bile acids and eventually excreted. Cholesterol that is part of the high-density lipoprotein molecule will not be deposited in blood vessel walls. Because of this, HDL is sometimes referred to as the "good" cholesterol and is believed to have a protective effect on the circulatory system (Moisio and Moisio, 1998).

2.3.4.1 Measurement of HDL-C
HDL-C is measured as cholesterol in the supernatant of samples following the precipitation of apo B-containing lipoproteins by several methods (Salam 2010).

Polyanion precipitation methods:
Polyanion precipitation was most commonly used to remove apo B-containing lipoproteins prior to analysis HDL-C. It required a sample pretreatment and was not fully automate. Most clinical laboratories have replaced precipitation techniques with automated homogeneous assay for HDL-C and LDL-C. HDL-C has been measured in the supernatant of samples following the precipitation of apo B-containing lipoproteins by polyanion (as heparin sulphate, dextranesulphate, phosphotungstate)-divalent cations (Ca2+, Mg2+ and Mn2+) (Salam, 2010).

Normal level:
Male: 29-62 mg/dl
Female: 34-82 mg/dl (Salam 2010).
2.4 Rationale:
Diabetes mellitus is one of the most serious problems worldwide, and there is strong association between lipid profile and Paternal history type 1 of Diabetes mellitus which is give a reason to be a target for researchers to find out new ways for early diagnosis, prevention, treatment and follow up. Many studies had conducted to determine the association between the lipid profile in Paternal history type 1 of diabetes mellitus in different parts of the world, but there are no such published studies in Sudan. Therefore this study is conducted to determine the association between and lipid profile in Sudanese with Paternal history type 1 of Diabetes mellitus in order to develop future prevention strategies for Diabetes mellitus within the target family.
2.5 Objective

2.5.1 General Objective
To access the lipid profile of type 1 Diabetic patients offspring.

2.5.2 Specific Objectives

- To estimate total cholesterol, triglyceride, HDL and LDL in study group.
- To compare lipid profile between offspring of diabetic and non-diabetic teenager.
- To detect the wariness of offspring about the diabetes.
- To detect psychological effect of type 1 diabetes in offspring.
- To study the sex effect type 1 diabetes in offspring lipid profile.
Chapter three
Materials and methods

3.1 Study design
This quantitative case control study

3.2 Study area and period
This study was conducted in Khartoum state during period from February to September 2014.

3.3 Study population
One hundred offspring their age ranged between 17-21 years were enrolled, fifty volunteers of offspring either father or mother with type1 diabetic patients. Where are fifty volunteers offspring of non-diabetic patient.

3.4 Inclusion criteria
Healthy off spring of type1 diabetic patients were selected as control in the study.

3.5 Exclusion criteria
Whose patient with DM type 1 and have other metabolic disease which may effect on result were excluded.

3.6 Ethical consideration
Ethical approval and an informed consent, aim and benefits of this study were explained to the participants.

3.7 Data collection and analysis
Demographic and clinical data were obtained from participants in this study by using medical record.

3.8 Sample collection
A local antiseptic (70% ethanol) was used to clean the skin, 3ml venous blood was taken from each participant by standard procedures, in lithium heparin container and then centrifuged at 3000 R.P.M for three minutes and clear plasma was separated in a plain containers and kept at -20c° until used

3.9 Statistical analysis:
The data was analyzed using Statistical Package for Social Sciences (SPSS) computer program version 11.5. Data were expressed as frequency, means ± standard deviation (SD); data analyzed using student independent t-test.
3.10 Measurement of plasma cholesterol

3.10.1 Principle
Under the action of cholesterol esterase to cholesterol esters resolve in cholesterol and Fatty acids, cholesterol oxidase the above mentioned cholesterol together with the loose cholesterol releasing cholesterol -3-one and hydrogen peroxide reacts with A phenol substitute and 4-aminoantipyrine (4AAP) to form red dye compound, the intensity of red color produced is directly proportional to total cholesterol in the sample when read at 520nm. (Salam 2010)

Procedure:

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<tr>
<td>Cholesterol Reagent</td>
<td>1ml</td>
<td>1ml</td>
<td>1ml</td>
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<tr>
<td>Distilled water</td>
<td>0.01ml</td>
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<tr>
<td>Standard</td>
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<td>Sample</td>
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Mixed, incubated for 10 min at 20-25°C and read sample and standard absorbance color was stable for 30minutes.

Calculation of cholesterol:

\[
\text{Absorbance of test} \times \frac{\text{Concentration of STD}}{\text{Absorbance of (STD)}}
\]

3.11 Measurement of Triglycerides

3.11.1 Principle
Triglycerides are broken down by lipase enzyme to glycerol and free fatty acids and in the presence of ATP, the glycerol is phosphorylated by glycerol kinase enzyme to glycerol-3-phosphate, and then the reaction can be completed by one of the following methods: glycerol-3-phosphate reduces NAD+ catalyzed by glucose-6-phosphate dehydrogenase enzyme to give dihydroxyacetone phosphate (DHAP), hydrogen ions and NADH that read at 340nm. glycerol-3-phosphate by an enzyme that called L-glycerophosphate oxidase enzyme gives dihydroxyacetone phosphate(DHAP) and hydrogen peroxide. The hydrogen peroxide is reduced by hydrogen peroxidase enzyme to water and oxygen that is received by oxygen
receptor (4-amino antipyrine), and in the presence of phenol as indicator, quinonimine red is formed and it is measured at 515nm green filter. (Salam, 2010)

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<td>Sample</td>
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Mixed, incubated for 10min at 20-25°C and read sample and standard absorbance color was stable for 60 minutes.

**Calculation:**

\[
\frac{\text{Absorbance of test}}{\text{Absorbance of (STD)}} \times \text{Concentration of STD}
\]

3.12 Measurement of plasma HDL-Cholesterol

**Principle:**

Very low density lipoprotein and low density lipoprotein in the sample precipitate with phosphotungstate and magnesium ions. The supernatant contains high density lipoprotein (HDL). The HDL cholesterol is then spectrophotometer measured by means of coupled reaction described above.

**Procedure:**

**Precipitation:**

<table>
<thead>
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<th></th>
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<th>Precipitate reagent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume</td>
<td>0.2 ml</td>
<td>0.5 ml</td>
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Mixed thoroughly and let stand for 10 min at room temperature.

Centrifuged at 400 rpm for 10 min

Reaction sample take from the supernatant.

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol Reagent</td>
<td>1ml</td>
<td>1ml</td>
<td>1ml</td>
</tr>
<tr>
<td>Distilled water</td>
<td>0.01ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard</td>
<td>0.01ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supernatant</td>
<td></td>
<td></td>
<td>0.01ml</td>
</tr>
</tbody>
</table>

Mixed, incubate for 10min at 20-25°C and read sample and standard absorbance color was stable for 60 minute.
Calculation of triglycerides:

\[
\frac{\text{Absorbance of test}}{\text{Absorbance of (STD)}} \times \text{Concentration of STD}
\]

3.13 Measurement of plasma LDLC:

**Friedewald’s equation:** \( \text{LDL} = \text{Total cholesterol} - (\text{HDL} + \text{VLDL}) \). (Salam, 2010)
3. Results

The population with relative age for both case and control expressed in M±SD as following Age (19.30 ± 2.706, 18.58 ± 1.465) and Wight (35.43 ± 4.847, 36.68± 9.208) and Height(1.3493 ± 0.217, 1.1484±1.1484) and Body mass index(20.56±5.919, 20.56 ±5.919) respectively

Table 4.1 shows Base line characteristic of study population

<table>
<thead>
<tr>
<th></th>
<th>M± SD Off spring of diabetic (N 50)</th>
<th>M± SD Off spring of non-diabetic (N 50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age(year)</td>
<td>19.30 ± 2.706</td>
<td>18.58 ± 1.465</td>
</tr>
<tr>
<td>Wight(kg)</td>
<td>35.43 ± 4.847</td>
<td>36.68± 9.208</td>
</tr>
<tr>
<td>Height(M)</td>
<td>1.349± 0.217</td>
<td>01.15±1.148</td>
</tr>
<tr>
<td>Body mass index</td>
<td>20.56 ±5.919</td>
<td>28.07 ±7.655</td>
</tr>
</tbody>
</table>
In this study showed highly significant deference in all lipid profile with exception of HDL between offspring of diabetic patients and non-diabetic person total cholesterol (169.8±1.99, 139.9±2.66), triglyceride (94±3.64, 127.5±3.45), LDL (106.8±2.06, 67.7±2.23) for off spring of diabetic and non-diabetic patients respectively with p value 0.00 for total cholesterol and LDL and 0.002 for triglyceride. while HDL showed no significant deference with (44.0 ± 1.04, 46.6±1.68) With P value 0.530 for off spring of diabetic and non-diabetic patients respectively.

Table 4.2 shows comparison between lipid profile in offspring of diabetic and non-diabetic patients

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean± SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol(mg/dl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient</td>
<td>169.8±1.99</td>
<td>0.000</td>
</tr>
<tr>
<td>Control</td>
<td>139.9±2.66</td>
<td></td>
</tr>
<tr>
<td>TG(mg/dl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient</td>
<td>94.9±3.64</td>
<td>0.002</td>
</tr>
<tr>
<td>Control</td>
<td>127.5±3.45</td>
<td></td>
</tr>
<tr>
<td>HDL(mg/dl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient</td>
<td>44.0±1.04</td>
<td>0.530</td>
</tr>
<tr>
<td>Control</td>
<td>46.6±1.68</td>
<td></td>
</tr>
<tr>
<td>LDL(mg/dl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient</td>
<td>106.8±2.06</td>
<td>0.000</td>
</tr>
<tr>
<td>Control</td>
<td>67.7±2.23</td>
<td></td>
</tr>
</tbody>
</table>
In this study showed no significant deference in all lipid profile between Diabetic maternal and Diabetic Paternal total cholesterol (175.44 ± 1.2198, 174.36 ± 1.328), triglyceride (88.50 ± 2.8178, 95.14 ± 3.537), HDL (88.50 ± 2.8178, 95.14 ± 3.5375), and LDL 126.18 ± 1.29681, 119.33 ± 1.8455) with P value 0.820 for total cholesterol, 0.572 for triglyceride, 0.282 for HDL and 0.245 for LDL for were and not ware.

**Table 4.3** shows comparison of lipid profiles between offspring of maternal and offspring of paternal diabetic patients

<table>
<thead>
<tr>
<th>Lipid profile</th>
<th>Diabetic maternal</th>
<th>Diabetic Paternal</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol(mg/dl)</td>
<td>175.4 ± 1.219</td>
<td>174.3 ± 1.328</td>
<td>0.820</td>
</tr>
<tr>
<td>TG(mg/dl)</td>
<td>88.50 ± 2.818</td>
<td>95.14 ± 3.538</td>
<td>0.572</td>
</tr>
<tr>
<td>HDL(mg/dl)</td>
<td>31.56 ± 0.924</td>
<td>36.00 ± 1.284</td>
<td>0.282</td>
</tr>
<tr>
<td>LDL(mg/dl)</td>
<td>126.2 ± 1.297</td>
<td>119.3 ± 1.846</td>
<td>0.245</td>
</tr>
</tbody>
</table>
Table 4.5 showed the awareness of offspring about the diabetic disease there was 20% are aware about the diabetic disease and 80% not Aware about diabetic disease.

<table>
<thead>
<tr>
<th>Aware</th>
<th>not aware</th>
</tr>
</thead>
<tbody>
<tr>
<td>20%</td>
<td>80%</td>
</tr>
</tbody>
</table>
Table 4.5 showed the impact of diabetic parental influence on offspring, with 52% expressing Emphasize, 12% Frustrated, and 36% Tolerance.

<table>
<thead>
<tr>
<th></th>
<th>Emphasize</th>
<th>Frustrated</th>
<th>Tolerance</th>
</tr>
</thead>
<tbody>
<tr>
<td>52%</td>
<td>12%</td>
<td>36%</td>
<td></td>
</tr>
</tbody>
</table>
4. Discussion, Conclusion and Recommendations:

5.1 Discussion

Diabetes mellitus describes a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defect in insulin secretion or action or both (Kattenbash, et al., 2011).

Recently, researchers emphasized in comparison of lipid profile in offspring of diabetic patients specially type1 diabetes mellitus, also changes in serum lipids metabolism strongly influence the early complication in diabetic patients which lead to cardiovascular disease. Few studies have been carried out on comparison of lipid profile between offspring of diabetic and non-diabetic patients, accordingly current study aims to evaluatelipid profile level in offspring of diabetic patientsin addition to study if there are difference between serum lipids levels in offspring of diabetic and non-diabetic patients to prevent early complication.

In the present study result showed thatcholesterol, triglycerides and High density lipoprotein were measured, Low density lipoprotein calculated according to standard formula in 50 patients with off spring of type 1 diabetic as group study, in addition to 50 off spring of healthy individual volunteers as control group.

The results of our study show that, lipid profile (include Cholesterol, Triglyceride HDL and LDL) were significantly increase in total cholesterol and low density lipoprotein LDL and significant decrease in triglycerides between offspring of diabetic and non-diabetic individual with P value (0.000, 0.002 and 0.000) respectively. These results are in agreement with previous study (Segovia et al., 2012) where no significant difference in high density lipoprotein HDL was observed.

Obviously there is no significant difference in all lipid profilebetween group of paternal diabetic and group of maternal diabetic offspring.

In our study observed that 80% of diabetic offspring population was not aware about diabetes mellitus disease, also more than fifty percent are emphasize from paternal diabetic disease.
5.2 Conclusion
In conclusion, lipid profile (include Cholesterol, Triglyceride HDL and LDL) were significant increase in total cholesterol and low density lipoprotein and significant decrease in triglycerides in offspring of diabetic patients compared with offspring of healthy parents.
5.3 Recommendations:

Lipid profile should be measured regularly in off spring of diabetic patients to avoid hypercholesterolemia which it is major cause of type 2 diabetes mellitus.

Increase the education rate about diabetes which reduces the incidence rate of gangrene and other complication of diabetes mellitus.

More research should be performed to determine the correlation between paternal history of diabetic patients and lipid profiles level with addition of other parameter.
References


SmamthP, Venkateswarulu M, Siva prabodh V. 2012 lipid profile in type 2 diabetes mellitus lipid profile level in type 2 diabetes mellitus from the Tribal population of Adilabad in Andhra Pradesh, Clinical and Diagnostic Research. 6(4):590-592