Sudden University of Science and Technology

College of Medical Laboratory Science

Clinical Chemistry Department

Assessment of Plasma Level of Total Cholesterol and LDL-Cholesterol in Patients with Type 2-Diabetes Mellitus

تقييم مستويات الكوليسترول الكلي والبروتينات الشحمية ذات الكثافة المنخفضة في مصل الدم لدى المصابين بالنوع الثاني من داء السكري

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الآية

قال تعالى :

(أَوَلَا يَذْكُرُ الْإِنسَانُ أَنَّا خَلَقْنَاهُ مِن قَبْلُ وَلَمْ يَكُ شَيْئًا)

صدق الله العظيم

سورة مريم الآية (67

Dedication

To

Our fathers

Our mothers

Sisters and brothers

To

Our teachers and especially our supervisor

To

Our colleagues whom help us

To

All people, whom we love, respect and appreciate.

Acknowledgements

Thank you our God, for giving us the ability and courage to bring this research to light. Our great thank to Dr. Mohammed Abd Elrhim our supervisor, who started with us this research from the zero level. He was the one who directed us to this important topic, so we are really grateful.

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We are also grateful to all my colleagues in the faculty of medical laboratory science –Sudan university, who stood firm behind us and gave us a great push forward especially all staff of clinical chemistry department.

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Abstract

Diabetes Mellitus is chronic metabolic disorder that is associated with cardiovascular complication of which the metabolic syndrome play prominent role lipid profile has been show to be an important predicator for metabolic disturbances including dyslipidaemia, diabetes mellitus and cardiovascular.

Across sectional study conducted during the period from march 2014 to august 2014 in Jaber Abu Allizz center , compared the plasma level of total cholesterol and LDL-cholesterol in type two diabetic patients . Forty diabetic patients 26 males and 14 females were compared to twenty normal healthy individuals as control group.

The level of plasma total cholesterol and LDL-cholesterol show significant increase in test group (p-value = 0.00) when compared with control group.

The study show insignificant difference between the mean of the level of plasma total cholesterol and LDL-cholesterol of test group in both sexes, The result of this study is conclude that the Diabetes Mellitus increase the plasma level of total cholesterol and LDL-cholesterol that lead to increase risk of cardiovascular disease compared to healthy group.

مستخلص الدراسة

مرض السكري هو خلل استقلابي مزمن يتعلق بمضاعفات امراض القلب التي يلعب فيها الاستقلاب دور بارز.الدهون لها تأثير في المشاكل الاستقلابية مثل مشاكل الدهون في مجرى الدم والسكري وإمراض القلب.

هذه الدراسة اجريت خلال الفترة ما بين مارس 2014 الى اغسطس 2014 حيث تمت مقارنة مستوى الكوليسترول الكلي والبروتينات ذات الكثافة المنخفضة في بلازما الدم عند 40 مريض بالسكري ،26 من الذكور و 14 من الاناث من مركز جابر ابو العز تمت مقارنتهم مع 20 من الاشخاص غير المصابين بالسكري كمجموعة تحكم.

بينت الدراسة ان مستوى الكوليسترول الكلي والبروتينات الشحمية ذات الكثافة المنخفضة في البلازما ان هنالك فروق ذات دلالة احصائية في مجموعة المرضى بالسكري بالمقارنة مع مجموعة التحكم .

واظهر انه لا يوجد فرقا ذو دلاله احصائيه بين متوسط الكوليسترول الكلي والبروتينات الشحمية ذات الكثافة المنخفضة في البلازما ان الذكور مقارنة مع الاناث ومن هذه الدراسة نستخلص ان مرض السكري يزيد من مستويات الكوليسترول والبروتين الدهني ذو الكثافة المنخفضة وذلك يزيد من خطر الاصابة بتصلب الشرايين مقارنة مع مجموعة الاصحاء.

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Abbreviations

CHD:	Coronary Heart Disease
FH:	Familial Hypercholesterolemia
GDM:	Gestational Diabetes Mellitus
HMG-COA:	3-hydroxy 3-methyl-glutaryl co enzyme A
IDL:	Intermediate Density Lipoprotein
IDDM:	Insulin Dependent Diabetes Mellitus
LDL:	Low Density Lipoprotein
NIDDM:	Non Insulin Dependent Diabetes Mellitus
VLDL:	Very Low Density Lipoprotein
WHO:	World Health Organization

Chapter One

Introduction and literature review

1 Introduction and Literature Review

1.1 Diabetes Mellitus

Diabetes Mellitus is group of metabolic disorder of carbohydrate metabolism in which glucose is underutilize, produce hyperglycemia and relative insulin deficiency, resistance or both. It affects more than120million by the year 2020. Diabetes is usually irreversible and although patients have reasonably normal life style, its late complication result in reduce life expectancy and major health costs (David *et al.*, 2005).

Diabetes is widely recognized as one of the leading cause's death. The rapid increase in diabetes parallels the increase in obesity and overweight. Recent information indicates that 5.5% in northern state of Sudan and 8.6% in Khartoum state have diabetes and the numbers are expected to rise (Elbagir *et al.*, 2008).

1.1.1Types of diabetes mellitus

In 1979, the national diabetes data group developed a classification and diagnosis scheme for diabetes mellitus. This scheme included dividing diabetes into two broad categories: Type1, insulin dependent diabetes mellitus (IDDM), Type2, Non insulin dependent diabetes mellitus (NIDDM).

Therefore the WHO guidelines recommend the following categories of diabetes: Type1 diabetes, Type 2 diabetes, other specific type and Gestational diabetes Mellitus (GDM) (Bishop *et al.*, 2010).

1.1.1.1Type 1 diabetes

Type 1 Diabetes is characterized by inappropriate hyperglycemia primarily a result of pancreatic islet B cell destruction and a tendency to ketoacidosis. Type 1 Diabetes mellitus is a result of cellular _mediated auto immune destruction of the pancreas, causing an absolute deficiency of insulin secretion .upper limit of 110 mg/dl on the fasting plasma glucose is designated as the upper limit of normal blood glucose. Type 1constitutes only 10% to 20% OF all cases of diabetes and commonly occur in child hood and adolescence .this disease is usually initiated by an environmental factor or infection (usually a virus) in individuals with genetic predisposition and causes the immune destruction of B cells of pancreas and, therefore, a decrease production of insulin.

Characteristics of Type 1 diabetes include abrupt onset, insulin dependence, and ketosis tendency. This diabetic type is genetically related. One or more of the following markers are found in 85% to 90% of individuals with fasting hyperglycemia: islet cell auto anti bodies, insulin auto antibodies, Glutamic acid decarboxylase auto antibodies, and tyrosine Phosphatase IA-2and IA-2B auto antibodies (Bishop *et al*.,2010).

Signs and symptoms include polydipsia (excessive thirst), polyphagia (increase food intake), polyuria (excessive urine production),rapid weight loss, hyperventilation ,mental confusion, and possible loss of consciousness(due to increase glucose to brain). Complications include microvascullar problems such as nephropathy , neuropathy, and retinopathy. Increased heart disease is also found in patients with diabetes (Bishop *et al*., 2010).

1.1.1.2 Type 2 diabetes

Type2 diabetes mellitus is characterized by hyperglycemia as a result of an individual's resistance to insulin with an insulin secretary defect. This resistance result in relative, not an absolute insulin deficiency.Type2 constitutes the majority of the diabetes cases. Most patients in this type are obese or have an increased percentage of body fat distribution in the abnormal region. This type of diabetes often goes undiagnosed for many years and is associated with a strong genetic predisposition, with patients at increased risk with an increase in age, obesity, and lack of physical exercise. Characteristic usually include adult onset of the disease and milder symptoms than in type1, with ketoacidosis seldom occurring. However, these patients are more likely to go into a hyperosmoler coma and are at an increased risk of developing macro vascular and micro vascular complications (Bishop *et al*., 2010).

1.1.1.3 Other specific types of diabetes

Other specific types of diabetes are associated with certain conditions (secondary), including genetic defect of Beta cells. function or insulin action, pancreatic disease, disease of endocrine origin, drug or chemical induced insulin receptor abnormalities, and certain genetic syndromes. The characteristics and prognosis of this form of diabetes depend on primary disorder (Bishop *et al*., 2010).

1.1.1.4 Gestational Diabetes Mellitus (GDM)

GDM is any degree of glucose intolerance with onset or first recognition during pregnancy. Causes of GDM include metabolic and hormonal changes. Patients with GDM frequently return to normal post partum. However, this disease is associated with increased prenatal complications and an increased risk for development of diabetes in later years. Infants born to mothers with diabetes are at increased risk for respiratory distress syndrome, hypocalcaemia, and hyperbilirubinemia. Fetal insulin secretion is stimulated in the neonate of mother with diabetes. However, when the infant is born and the umbilical cord is severed, the infant's over supply of glucose is abruptly terminated, causing sever hypoglycemia (Bishop *et al*., 2010).

1.1.3 Insulin

Insulin is primary hormone responsible for the entry of glucose into the cell, it is synthesized by the Beta cells of islets of Langerhans in the pancreas. When these cells defect an increase in body glucose, they release of insulin causes an increased movement of glucose into the cells and increased glucose metabolism. Insulin is normally released when glucose levels are high and is not released when glucose levels are decreased. It decreases plasma glucose levels by increasing the transport entry of glucose in muscle and a dispose tissue by way of non specific receptors. it also regulates glucose by increasing glycogensis, lipogenesis and glycolysis and inhibiting glycogenolysis. Insulin is the only hormone that decreases glucose levels and can be referred to as a hypoglycemic agent (Bishop *et al*., 2010).

1.2 Plasma Lipids

The term lipid represents a large group of compounds that are virtually insoluble in water .This group can be separated into four primary groups (Cholesterol and its esters, glycerol esters, fatty acids and phospholipids). Among these, cholesterol and triglycerides are the two most prominent lipids found in serum (Bishop *et al*., 2010).

1.2.1 Cholesterol

Is an unsaturated steroid alcohol of high molecular weight, consisting of aperhydrocyclopentanthroline ring and aside chain of eight carbon

atoms. In its esterifies form, it contain one fatty acid molecule. Cholesterol is found almost exclusively in animals .Virtually all cells and body fluids contain some cholesterol (Bishop *et al.,* 2010).

It has a molecular weight of 386 Da and contains 27carbon atoms of which 17 are incorporated into four fused rings (John Baynes *et al.,* 1999).

Cholesterol is used for the manufacture and repair of cell membrane for synthesis of bile acids and vitamin D, and is the precursor of five major classes of steroid hormones, progestin, glucocorticoids, mineral corticoids, androgens and estrogens (Bishop *et al.,* 2010).

1.2.1.1Synthesis

The liver is the major side of cholesterol synthesis. Although cholesterol is formed from acetyl-coA are condensed to produce 3-hydroxy3-methyl-glutaryl coenzyme A (HMG-CoA), which, in Turn, is converted to mevalonic acid which converted into squalene after series of condensation and rearrangement steps. Sequence then cyclizes to form lanosterol, which is further modified to yield cholesterol (Bishop *et al.,* 2010).

1.2.1.2 Metabolism and excretion

Cholesterol cannot be metabolized by mammalian cells and cannot be digested in the gut into carbon dioxide and water removal from the body is thus dependant on to excretion via the feces. as the section on bile acids indicated there is considerable flux of cholesterol, either directly or as bile acid metabolites from the liver into duodenum via the common bile duct about 1g of cholesterol is eliminated from the body through the feces, approximately 50% is excreted after conversion to bile acids. The remainder is excreted as the isomeric saturated neutral sterols [coprostanol (5B) and cholesterol (5a)

produced by bacterial reduction of cholesterol molecules] (Baynes *et al* 1999).

1.2.1.3 Clinical Significance

1.2.1.3.1 Hypercholesterolemia

Is the presence of abnormally high levels of cholesterol in blood possible causes are obesity, Diabetes mellitus and insulin resistance, excess alcohol intake, nephritic syndrome and hypothyroidism.

is the lipid abnormality most closely linked to heart disease, one form of the disease, which is associated with genetic abnormalities that predispose affected individuals to elevated cholesterol levels ,is called familial hypercholesterolemia (FH). Homozygote for FH are fortunately rare but can have total cholesterol concentrations as high as 800mg /dl to 1000mg/dl, and patients frequently have their first heart attack, while still in their teen age years. Heterozygote for the disease are seen much more frequently, because the disease caused by an autosomal co dominant disorder. Patients tend to have total cholesterol concentration in the range of 300mg/dl to 600mg/dl and if not treated, will become symptomatic for heart disease in their 20s to50s. Approximately 5% of patients younger than age 50 years with CHD are FH heterozygote (Bayne *et al*, 1999).

1.2.1.3.2 Hypo cholesterolemia

Is the presence of abnormally low (hypo) levels of cholesterol in blood. Possible causes of low cholesterol are statins, hyperthyroidism, adrenal insufficiency, liver disease, malabsorption (celiac disease), malnutrition,

Beta lipoproteinemia, manganese deficiency, smith-lemli-opitz syndrome, marfan syndrome and leukemia (John Bayne *et al.*, 1999).

1.2.2 Low density lipoproteins Cholesterol (LDL-c)

Is the principal carries of cholesterol, mainly in the form of cholesterol "esters". Each native LDL particle contain a single abo lipoprotein B-100 molecules (Apo-100,aprotein with 4536 amino acid residues) which circulates the fatty acids, keeping them soluble in the aqueous environment(Juutilainen A *et al.*, 2004).

1.2.2.1 Metabolism of LDL-c

LDL is formed from VLDL via IDL .In normal circumstances IDL is further degraded by hepatic lipoprotein lipase to form LDL, LDL catabolism take place in the liver and the peripheral tissue (Udawat *et al.,* 2001).

LDL interact with high affinity receptor sites located in regions of cell membrane called coated pits, the bound LDL is thin internalized by invagination of these pits into the cell, where the pitds pinch off to form endocytotic vesicles. These vesicles fuse with intracellular lysosomes and the LDL is subjected to series of hydrolyzed by lysosomal cholesterol esterase and the free cholesterol enters the cytoplasm. The release of free cholesterol is responsible for regularity responses that assess is cholesterol homeostasis (Udawat *et al.*, 2001).

Most of the plasma LDL-c is removed by LDL receptors (Talat N *et al.*, 2003).

1.3 Objectives

1.3.1General Objective

To assess the total cholesterol and LDL–c in type-2 diabetes mellitus patients in Jabir Abulizz center for diabetes mellitus .

1.3.2Specific objectives

1. To measure the plasma total cholesterol and LDL –c level in the test groups compared with control group.
2. To measure the relationship between plasma total cholesterol and LDL –c level and sex of patients.
3. To correlate between plasma total cholesterol and LDL –c level and duration of diabetes.

Chapter Two

Material and Methods

2 Materials and Methods

2.1 Materials

2.1.1 Study Design

Cross sectional hospital based study.

2.1.2 Study period and area:

The study will be conducted in Jaber Abulizz during the period of March 2014 to august 2014.

2.1.3 Study population

Patients with type 2 diabetes mellitus were enrolled in this study as case group and volunteer non diabetic as control group.

2.1.4 Inclusion criteria

Patients of type 2 diabetes mellitus were included to this study.

2.1.5 Exclusion criteria

Patients with hypertension, thyroid disorder, renal disease, gout and coronary heart disease will be excluded.

2.1.6 Data collection and clinical assessment

Specially designed questionnaire and interview with the patient well be made.

Doctor will conduct the clinical examination and assessment.

2.1.7 Sample Size

Forty adult diabetic patients with type 2 diabetes will be included in this study and apparently health subject used as control.

2.1.8 Sampling

Fasting blood sample will be taken from the participants and will be preserved in containers used lithium heparin and after centrifugation the plasma will be used to measure level of cholesterol and LDL – cholesterol in diabetes.

2.1.9 Ethical consideration

Permission of this study was obtained from the local authorities in the area of the study. The objectives of the study were explained to all individuals participating in this study.

2.2 Methods

2.2.1 Estimation of cholesterol

Principle

In the presence of cholesterol esterase the cholesterol ester in the sample are hydrolyzed to free cholesterol and fatty acid, the free cholesterol produced in oxidized by cholesterol oxidase to give cholestene -3-one and hydrogen peroxide. The presence of hydrogen peroxide and oxygen acceptor (phenol and 4- aminophenazone) the hydrogen peroxide give red color quinonamine which is proportional to the amount of cholesterol in sample**.**

Procedure: appendices

Reagents: appendices

Calculation: appendices

Reference Range: appendices

2.2.2 Estimation of LDL

Principle

LDL precipitated by poly- venial sulphate (buffered by poly-ethylene-glycol PH 5.2) after centrifugation, chylomicrons ,VLDL ,HDL , "other lipoproteins than LDL" are remain in supernatant and cholesterol of them estimated by cholesterol oxidase method , and after estimation of total cholesterol also , LDL cholesterol can be calculated as follow:

LDL cholesterol = total cholesterol concentration –other lipoprotein cholesterol.

Sample: un-haemolyzed serum or heparinized plasma preferable fasting.

Procedure: appendices

Reagents: appendices

Calculation: appendices

Reference Range: appendices

Quality control

It recommended to use the lipid control serum level I and II to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

2.2.3 Data analysis

Data was analyzed by using statistical package for social science (SPSS) computer system program. The means and standard deviation (SD) of plasma levels of cholesterol and LDL- cholesterol were calculate and t-test was used for comparison (significant at P-value ≤ 0.05). Linear regression analysis was used to assess correlation between duration and level cholesterol and LDL-cholesterol.

Chapter Three

Result

3 Result

The level of total cholesterol and LDL-cholesterol were monitored in 40 diabetes patient type2 in order to see the impact of disease controlled diabetes mellitus on the level of total cholesterol and LDL-cholesterol.

The result obtain were as follow:

Table (3-1) shows significant increase in level of cholesterol in patients compared with control (235.4 ± 75.6) and (116.5 ± 35.17) respectively with *P*-value is (0.00).

Table (3-2) shows significant increase of level of LDL-cholesterol in patients compared with control (42.2 ± 16.4) and (21.3 ± 9.5) respectively with *P*-value is (0.00).

Table (3-3) shows insignificant difference in level of cholesterol when compared with males and females in test group (234.8 ± 81.4) and (236.5 ± 66) with *P*-value (0.94).

Table (3-4) shows insignificant difference in level of LDL when compared with males and females in test group(40±18) and (45±11.8) with *P*-value (0.3).

Figure (3-1) shows LDL concentration of test group had negative correlation with duration (r = -0.213, *P*-value = 0.186).

Figure (3-2) shows cholesterol concentration of test group had positive correlation with duration (r=0.094, *P*-value = 0.564) .

Table (3-1) comparison between the mean and standard deviation of cholesterol of test group and control group.

	Test group No=40	Control group No=20	*p*-value
Cholesterol (mg\dl)	235.4 ± 75.6	116.5 ± 35.17	0.00

Table (3-2) comparison between the mean and standard deviation of LDL-cholesterol of test group and control group.

	Test group No = 40	Control group No =20	P = value
LDL (mg\dl)	42.2±16.4	21.3±9.5	0.00

Table (3-3) comparison between the mean and standard deviation of cholesterol level of male and female.

	Male No=26	Female No=14	*P*-value
Cholesterol (mg\dl)	234.8±81.4	236.5±66	0.947

Table (3-4) comparison between the mean and standard deviation of LDL-cholesterol level of male and female.

	Male No=26	Female No=14	*P*-value
LDL (mg\dl)	40±18	45±11,8	0.3

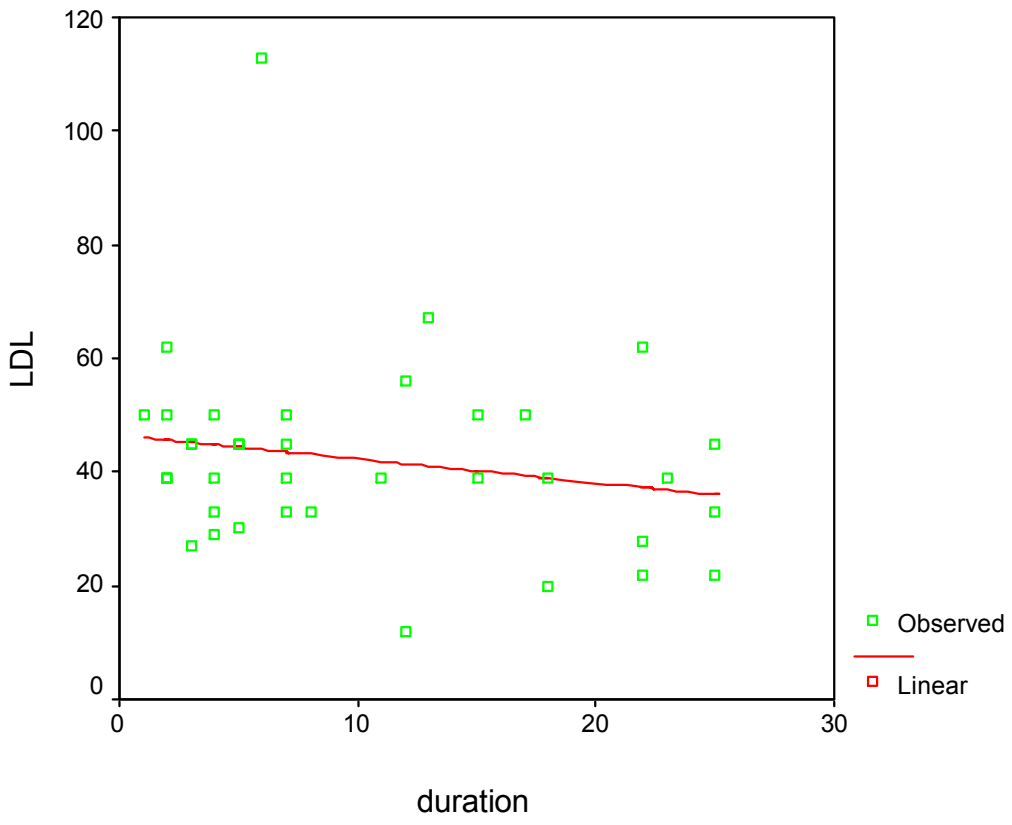

Figure (3-1) correlation between duration and concentration level of LDL of test group.

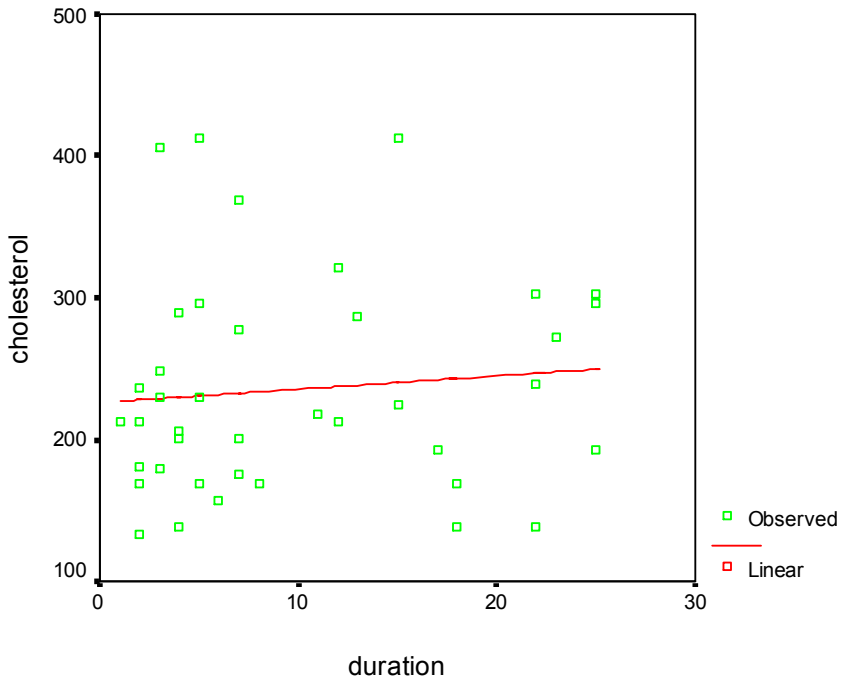

Figure (3-2) the correlation between duration and concentration level of cholesterol of test group.

Chapter Four

Discussion, Conclusion and Recommendation

4 Discussion, Conclusion And Recommendations

4.1 Discussion

Diabetes mellitus is chronic metabolic disorder that is associated with cardiovascular complication of which the metabolic syndrome play prominent role lipid profile has been show to be an important predicator for metabolic disturbances including dyslipidaemia, diabetes mellitus and cardiovascular.

In this study total cholesterol and LDL-cholesterol were measured in 40 patients of type2 diabetes, 26 male (40-80years) and 14 female (40-80 years) compared with 20 healthy individuals as control group.

The results of the present study provided experimental evidence that The study found significant increase in the mean of plasma total cholesterol in the test group when compared with control group (*P*-value = 0.000), in addition to that there is significant increase in the mean of plasma LDL-cholesterol in the test group when compared with the control group (*P*-value= 0.000). The results found insignificant difference in the mean of plasma total cholesterol and LDL-cholesterol when compared with male and female in test group (*p*- value = 0.947) and (*p*- value=0.3) respectively. This study agree with study done by Dormandy, who found that there is significant increase in the mean of plasma total cholesterol and LDL –cholesterol in test group when compared with control group and insignificant difference when compared with male and female among test group (Dormandy *et al*., 2005).

There is weak positive correlation between level of cholesterol and duration of diseases.

there is weak negative correlation between level of LDL-cholesterol and duration of diseases.

4.2 conclusion

From the results of this study it is conclude that:

1- the level of plasma total cholesterol and LDL- cholesterol in the type 2 diabetes patients is higher than those of control which increase the risk of cardiovascular disease.

2- the level of cholesterol and LDL-cholesterol are not effect by gender.

3- There is weak positive correlation between level of cholesterol and duration of dieses.

4.There is weak negative correlation between level of LDL-cholesterol and duration of dieses.

4.3 Recommendations

1- Patient with type 2 diabetes mellitus should be routinely checked for their plasma lipids every three month.

2- Further studies on large sample size should be performed. 3- Further investigation should be conducted to study the lipid profile (HDL –cholesterol, triglyceride) in diabetic patient – type2.

4- health education, diet control and exercise are important factors in lowering the body weigh especially in obese patient whose suggest to be cardiovascular and cerebrovascular complication.

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Appendices

Questionnaire

A. General information

1.Name…….. 2.Age……years

3.Gender (a).male( ) (b)female ( ) 4.Occupation……

B. Clinical information

1.Duration of disease……years

2.preasent history of disease:

Gout……

Hypertention…….

Renal disease…….

Thyroid disease…….

Familial Hyperlipidaemia……

Coronary Heart disease…….

3.Treatment: Hypoglycemic Agent ( ) Diet ( ) Non ( )

C. Biochemical Measurement:

1.Total Cholesterol ……mg/dl

2.LDL-Cholesterol……mg/dl