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PROFILES OF LIVER FUNCTION TESTS AMONG TYPE 2 DIABETIC PATIENTS WHO ARE RECEIVING DIFFERENT ANTI-DIABETIC DRUGS ATTENDING TIKUR ANBESSA SPECIALIZED HOSPITAL.

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A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES OF ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN MEDICAL BIOCHEMISTRY.

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This is to certify that the thesis prepared by Desaleng Dango entitled: Profiles of Liver Function Tests Among Type 2 Diabetic Patients Who are Receiving Different Anti- Diabetic Drugs Attending Tikur Anbessa Specialized Hospital, Addis Ababa and Submitted in the Partial Fulfillment of the Requirements for the Degree of Master of Science (Medical Biochemistry) complies with regulation of the university and meets the accepted standards with respect to originality and quality.

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Declaration

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Table of contents page

List of figure...... viii

List of Table..... viii

List of abbreviations and acronyms ……..……...……...……X

Acknowledgment……...…....xi

Abstract ……..……..……...……xii

1. Introduction ……..…….……1

1.1 Back ground information……..……1

1.2 Statement of problem…….……3

2. Literature Review………..5

2.1 Type 2 diabetes mellitus……..…. …5

2.2 Fatty liver disease…….…….. ….6

2.3 Non alcoholic fatty liver disease……...…….....7

2.4 Alcoholic liver disease ; ……...…….……...….9

2.5 Liver and diabetes ……...……...…...…….11

2.6 Hepatic carcinogenesis……...……..…..…12

2.7 Viral hepatitis ……...…..…… ……13

2.8 Hepatogenous diabetes……...……..……..14

2.9 Management of type 2 Diabetic mellitus……..……..15

2.9.1 Metformin…….……16

2.9.2 Sulfonylureas…….,,,,……17

2.9.3 Insulin…….,..……19

2.10 Significance of the study……..……..,…19

2.11. Hypothesis of the study……..…….……..20

3. Objectives of the study…….……20

3.1 General Objective of the study…….…….……..20

3.2. Specific Objective……...……20

4. Materials and Methods ……..21

4.1 Study area……...…..….21

4.2. Study design and period……..21

4.3. Source of population……...…22

3.4. Study population……..……...……..…22

4.5 Inclusion and Exclusion criteria……...……22

4.5.1 Inclusion criteria……...……..……..…22

4.5.2 Exclusion criteria……...……..…….22

4.6 Sampling technique and sample size determination……23

4.7. Study variables…….…….……..……24

4, 7.1 Independent variable…….…….……..24

4.7.2 Dependent variable…….…….…….24

4.8. Data Analysis…….……....…….24

4.9. Data Quality assurance……...……25

4.10. Ethical consideration ……..……..25

4.11 Method of Data collection and Analysis…….……..…….....26

4.11.1 Blood collection …….……..…….....26

4.11.2 Body Mass Index (BMI)……..……...……26

4.12 Laboratory Testing Methods…….…….……27

4.12.1 Serum HDL determination methods……...……..……..27

4.12.2 Colorimetric determination of total cholestrol……29

4.12.3. Serum triacylglycerol assay……...……31

4.12.4 LDL-cholesterol ……..33

4.12.5 Alanine Aminotransferase (ALT) assay method..…….…….……33

4.12.6 Aspartate Aminotransferase (AST) assay method..……. …….....35

4.12.7 Total bilirubin determination method...…….…….37

4.12.8 Alkaline phosphatase assay method…….…...38

4.12.9 Total Protein determination method. ……..…...39

4.12.10 Albumin determination method.……..…….……40

4.12.11 Fasting blood glucose determination……...…….40

5. Result……...……'…….……41

6. Discussion……...……...……56

7. Conclusion ……...……63

8. Limitations and Recommendation …… ……64

Reference…...……...……...….65

Appendix..... 75

Annex I: Consent form in English.....75

Annex 2: Consent form in Amharic.....78

Annex 3:- Sample collection sheet.....81

Annex 4:- Questionnaire…….....82

Annex 5:- Questionnaire in Amharic ……..……..……84

List of figures Page

Figure 1:- The correlation of BMI with AST of patients. 57

Figure 2:-Correlation of age with total cholesterol of patients. 58

Figure 3:- Correlation of diabetic duration with ALT of patients. 58

List of Tables page

Table 1:- Classification of Overweight and Obesity by BMI……...….26

Table 2:- Demographic and clinical characteristics of type 2 diabetic patients who are receiving different anti-diabetic drugs and diabetic patients who don't receive any medication (Controls)……..42

Table 3:- Mean values of different parameters in patients receiving insulin and insulin plus metformin…….……..43

Table 4:- Mean values of different parameters in patients receiving insulin and metformin……...……44

Table 5:- Mean values of different parameters in patients receiving insulin and metformin plus glibenclamide…….……45

Table 6:- Mean values of different parameters in patients receiving insulin and glibenclamide…….46

Table 7:- Mean values of different parameters in patients receiving metformin and glibenclamide……...……..47

Table 8:- Mean values of different parameters in patients receiving metformin and metformin plus glibenclamide…….48

Table 9:- Mean values of different parameters in patients receiving metformin and insulin plus metformin. ……..……49

Table 10:- Mean values of different parameters in patients receiving insulin plus metformin and metformin plus glibenclamide……..…….50

Table 11:- Mean values of different parameters in patients receiving metformin plus glibenclamide and glibenclamide……..…….51

Table 12:- Mean values of the biochemical parameters in tye 2 diabetic patients who are receiving anti-diabetic drugs and control group. ……..……52

List of Abbreviations and Acronyms

ADA……American Diabetic Association

ALT……. Alanine aminotransferase

AST…….Aspartate aminotransferase

BMI……..Body Mass Index

DM…….. Diabetes Mellitus

FBS……..…..Fasting Blood Sugar

FPG……..…..Fasting Plasma Glucose

IDA……...International Diabetic Association

HDL……High-densitylipoprotein(HDL)-cholesterol

IDF.....International Diabetic Foundation

IDDM.....Insulin Dependent Diabetes Association Mellitus

IDF…..…….International Diabetic Foundation

LDL……..…Low-density lipoprotein

LFT……..Liver function tests

LPL……. Lipoprotein lipase

MeS……...Metabolic syndrome

NIDDM.....Non-insulin Dependent Diabetic Mellitus

SST……. Serum separator tube

TC……...Total cholesterol

WHO……..World Health Organization

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Abstract

Background: - Diabetes Mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia with disturbances of carbohydrates, fat and protein metabolism resulting from insufficient insulin secretion, defects in insulin secretion, insulin action, or both. Individuals with type 2 diabetes have a higher incidence of liver function abnormalities. In this study we assess liver function tests in patients with type 2 diabetes mellitus who are receiving different anti- diabetic drugs and we examine factors associated with these biochemical changes.

Objective: - To investigate profile of liver function tests among type 2 diabetic patients who are receiving different anti-diabetic drugs attending Tikur Anbessa Specialized Hospital.

Methods: - Hospital based cross-sectional study was conducted on 70 type 2 diabetic patients who are receiving different anti-diabetic drugs and 35 type 2 diabetic patients who do not receive any medication were recruited for this study. The blood was taken at the fasting period and liver enzymes, total protein (TP), albumin (AL), total bilirubin (TB), fasting blood sugar (FBS), lipid profiles and body mass index (BMI) were carried out in all patients and control group following the standard procedures.

Results: Mean values of alkaline phosphatase (ALP), albumin (AL), TP and FBS were significantly higher in type 2 diabetic patients receiving different anti-diabetic drugs than in control group. In contrast, mean value of Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) among study group were lower than control group. Mean values of TC and LDL were lower in study group than in the control group. The mean value differences between the study group and control of TB, TG and HDL were statistically not significant. There are no significant differences of liver enzymes, TP, AL, TB, and lipid profiles in different patients who were on different anti-diabetic drugs. But mean value of liver enzymes and lipid profiles were slightly lowered in patients receiving mono therapy of insulin and metformin than insulin plus metformin, whereas BMI and FBS were lowered in their combination therapy receiving group. Similarly mean value of FBS, ALT, TC, HDL, LDL and lipid profiles were lowered in patients receiving mono therapy of glibenclamide and metformin than glibenclamide plus metformin combination therapy receiving group, while BMI and TB were increased in patients receiving mono therapy of metformin and glibenclamide.

Conclusion: The anti-diabetic drugs were found to have an effect in lowering liver enzymes and lipid profiles in type 2 diabetic patients. The different biochemical parameters tested were more or less similar in different groups of individuals who were on different anti-diabetic drugs of mono therapy or combination therapy.

Keywords: - LFT; Alkaline phosphatase; Lipid profile; Aminotransferases (ALT & AST)

1. Introduction

1.1 Background information

Diabetes Mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia with disturbances of carbohydrates, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. Glucose is an important regulator of various pancreatic β-cell processes, including insulin biosynthesis and release. Glucose, over short intervals, stimulates insulin biosynthesis at the level of translation. Glucose thus becomes the final common pathway for the transport of almost all carbohydrates to tissue cells. Normally, rates of glucose influx into the circulation and those of glucose efflux out of the circulation into tissues other than the brain are coordinately regulated largely by the plasma glucose lowering hormone, insulin, and the plasma glucose raising hormones, glucagon and epinephrine. Thus systemic glucose balance is maintained, hypoglycemia as well as hyperglycemia is prevented, and a continuous supply of glucose to the brain is ensured [1-3]

The prevalence of diabetes has reached epidemic proportions. The African region is expected to experience the highest increase in coming years with estimated increase in prevalence rates of 98% for sub-Saharan Africa, and 94% for North Africa and the Middle East [4].

The IDF Atlas 6th edition 2013 report (ARF) revealed that in 2013, 19.8 million adults in the Africa region are estimated to have diabetes, with a regional prevalence of 4.9%. The top four countries (Nigeria 3.9 million, South Africa 2.6, Ethiopia 1.9 million and United Republic of Tanzania 1.7) with the highest number of people with diabetes make up just over half of the total number in the region. This would rise to 41.5 million by 2035 with prevalence of 6 %, an increase of 109.6 %, as such exceeding the predicted worldwide increase of 55% [5].

When it is not prevented and properly managed diabetes is one of the major causes of premature illness and death worldwide. Non- communicable diseases including diabetes account for 60% of all deaths worldwide and more than 80% of diabetes deaths occur in low- and middle-income countries caused 5.1 million deaths in 2013 globally[4]. Statistics for medical complications from diabetes are also concerning. Proportions of patients with diabetic complications in sub Saharan region ranged from 7-63% for retinopathy, 27-66% for neuropathy, and 10-83% for nephropathy [6].

There exists an association between diabetes and liver injury. Liver plays a major role in the regulation of carbohydrate homeostasis. Hepatocellular glycogen accumulation leads to hepatomegaly and liver enzyme abnormalities in poorly controlled diabetes patients. In hyperglycemic states, there will be intracellular glycogen accumulation in the hepatocytes due to increased glycogen synthesis, causing typical biochemical findings of mild to moderately elevated aminotransferases, normal liver synthetic function, with or without mild elevations of alkaline phosphatase [7].

Liver disease is an important cause of death in type 2 diabetes. In the population-based Verona Diabetes Study, cirrhosis was the fourth leading cause of death and accounted for 4.4% of diabetes related deaths [8].

Liver function tests (LFTs) are commonly used in clinical practice to screen for liver disease, monitor the progression of known disease, and monitor the effects of potentially hepatotoxic drugs. The most common LFTs include the serum aminotransferases alkaline phosphatase, bilirubin, albumin, and prothrombin time. Aminotransferases, such as alanine amino transferase (ALT) and aspartate aminotransferase (AST), serve as a marker of hepatocyte injury. Alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), and bilirubin are markers of biliary

function and cholestasis. Chronic mild elevation of transaminases is frequently found in type-2 diabetic patients. Diabetes mellitus (DM) is associated with non-alcoholic fatty liver disease (NAFLD) including its severe form, non-alcoholic steatohepatitis (NASH). Among patients with diabetes, the risk of chronic liver disease is doubled, independent of alcoholic or viral hepatitis [9].

In another prospective cohort study, cirrhosis accounted for 12.5% of deaths in patients with diabetes [10]. Diabetes, by most estimates, is now the most common cause of liver disease in the U.S. Cryptogenic cirrhosis, of which diabetes is, by far, the most common cause, has become the third leading indication for liver transplantation in the U.S. [11]. Virtually the entire spectrum of liver disease is seen in patients with type 2 diabetes. This includes abnormal liver enzymes, nonalcoholic fatty liver disease (NAFLD), hepatocellular carcinoma, and acute liver failure. Finally, the prevalence of diabetes in cirrhosis is 12.3–57% [12].

1.2 Statement of problem

Type 2 diabetes is present in the range of 85-95% of all diabetes cases in high-income countries. In Ethiopia it has been reported as number of cases of diabetes to be estimated about 1.9 million in 2013 [5].

Individuals with type 2 diabetes have a higher incidence of liver function test abnormalities than non diabetics. Mild chronic elevations of transaminases often reflect underlying insulin resistance. The excess free fatty acid found in the insulin-resistant states is directly toxic to hepatocytes. Putative mechanisms include liver cell membrane disruption at high concentration of fatty acids, mitochondrial dysfunction, toxin formation, and activation and inhibition of key steps in hepatic metabolism. Several oral hypoglycemic such as sulphonylureas, biguanide,

meglitinides, pioglitazone and α-glucosidase inhibitors drugs are used at present for treatment of type 2 diabetes mellitus [8].

There is a link between liver function abnormality progression and type 2 diabetic mellitus. The cause of such liver function abnormality may varies; such as NALD, alcohol, viral infection and etc. The severity of type 2 diabetes and severity of liver function abnormality influence the therapy. So patients receiving different anti-diabetic drugs may improve this abnormality [13].

Lebovitz *et al.,* [15] have reported that there was no difference in the incidence of liver abnormalities in patients treated with rosiglitazone, placebo, metformin, or a sulfonylurea in trials involving 5,000 patients.

Rivellese *et al.,*[16] found that insulin therapy compared with glibenclamide is associated with greater decreases in plasma triglyceride, very low density lipoprotein , increases in the high density lipoprotein and no change in low density lipoprotein.

Studies have shown that diabetes mellitus is a progressive disorder which cannot be effectively managed with drug mono therapy. Regardless of drug management, the pancreatic beta-cells in type 2 diabetic patients continue to deteriorate leading to worsening glycemic control and consequent requirement for multiple therapies or exogenous insulin [17].

However, In Ethiopia, no study was undertaken to examine the extent of compliance and adherence to progression of liver function tests among T2DM patients who are receiving different anti-diabetic drugs. For that reason, this study was undertaken to fill this gap in the literature. The present study is expected to investigate the causative link of liver function abnormality progression in T2DM patients who are receiving different anti diabetic drug regimen at Tikur Anbessa Specialized Hospital.

2.0 Literature Review

The 6th edition of the IDF Diabetes Atlas confirms the precipitous rise in diabetes over the last few years. In 2013, 382 million people estimated to have diabetes, with dramatic increases seen in countries all over the world. The overwhelming burden of the disease continues to be shouldered by low- and middle-income countries. Socially and economically disadvantaged people in every country carry the greatest burden of diabetes and are often the most affected financially. Africa has the highest proportion of undiagnosed diabetes which is about 63 per cent; This is according to a report by the International Diabetes Federation (IDF) published in the world diabetes Atlas. It said 522,600 people in the Region died from diabetes-related causes in 2013. This represents 8.6% of deaths from all causes in adults [5].

2.1 Type 2 diabetes mellitus

Concepts regarding diabetes are changing. Until recently, diabetes was classified into type 1 and type 2 diabetes, with several other types of diabetes, including gestational diabetes, drug-induced diabetes and others also being defined [18]. Type1 diabetes occurs mainly in children, involves autoimmune destruction of pancreatic islet beta-cells, and renders patients completely dependent upon insulin. Type 2 diabetes initially involves insulin resistance, leading to hyperinsulinemia, and is associated with obesity and metabolic syndrome, but eventually progresses to involve pancreatic beta cell dysfunction with an insulin deficit and most diabetics (over 90%) are type 2 diabetics [19].

Presumably T2DM develops when a diabetogenic lifestyle (excessive caloric intake, inadequate caloric expenditure, obesity) is superimposed upon a susceptible genotype. About 90% of patients who develop T2DM are obese. The major risk factors for type 2 DM are the following:

age greater than 45 years (though as noted above, type2 diabetes is occurring with increasing frequency in young individuals), weight greater than 120% of desirable body weight. Family history of type 2 diabetes in first degree relative (e.g. parent or sibling), hypertension (>140/90 mmHg) or dyslipidemia (high density lipoprotein choletrol level < 40 mg/dl or triglyceride level > 150 mg/dl), history of gestational diabetes mellitus or of delivering a baby with a birth weight of > lb[3].

Diabetes mellitus is one of the leading causes of morbidity and mortality in United States because of its role in development of cardiovascular, neuropathic, and retinal disease [20].

The risk of coronary heart disease is 2-4 times greater in patients with diabetes than in individual without diabetes. Cardiovascular disease is the major source of mortality in patients with T2DM. Approximately two third of people with diabetes die of heart disease or stroke[3].

Major symptoms are polyuria, polydipsia, polyphagia, weight loss. Other symptoms that might suggest hyperglycemia include blurred vision, lower extremity paresthesiasis. However, many patients with T2DM are asymptomatic and their disease remains undiagnosed for many years [3].

2.2 Fatty liver disease

Fatty liver disease (FLD), whether it is alcoholic fatty liver disease (AFLD) or nonalcoholic fatty liver disease (NAFLD), encompasses a morphological spectrum consisting of hepatic steatosis (fatty liver). Several mechanisms may lead to a fatty liver: (1) increased free fatty acids supply due to increased lipolysis from both visceral/subcutaneous adipose tissue and/or increased intake of dietary fat; (2) decreased free fatty oxidation; (3) increased de novo hepatic lipogenesis (DNL) and (4) decreased hepatic very low density lipoprotein–triglyceride secretion. Free fatty acid delivery to the liver accounts for almost two-thirds of its lipid accumulation [21].

Moreover, fat accumulation in the liver is, independent of body mass index and intra abdominal and overall obesity, characterized by several features of insulin resistance in normal weight and moderately overweight subjects. Although NAFLD/NASH is generally considered as the result of insulin resistance syndrome including obesity, T2DM and hyperlipidemia, some recent studies implicate that NAFLD could also be a pre-diabetic condition [22]. For example, Fan *et al.,* [23] conducted a retrospective study on a cohort of 358 individuals with hepatic ultrasound defined fatty liver and 788 age, sex and occupation matched controls for 4-7 years, which showed that metabolic syndrome components were present at a greater frequency among those with fatty liver than among controls.

2.3 Non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD) was first recognized in 1980. Over the past few decades, it has rapidly become the most common form of liver disease, concomitant with the increasing prevalence of obesity worldwide [24].

A long-term high fat diet is a risk factor for insulin resistance and type 2 diabetes mellitus (T2DM), which are characterized by elevated levels of serum free fatty acids (FFAs), declined oxidation of intracellular FFAs and increased fat accumulation in the target organs of insulin [25]. Fat accumulation in the liver may result in non-alcoholic fatty liver disease (NAFLD). Currently, a large number of people showed excessive fat accumulation in the liver. NAFLD affects 20%-40% of the population, and approximately 30% of the patients with NAFLD progress to nonalcoholic steatohepatitis (NASH) [26].

T2DM and NAFLD (T2DM-NAFLD) are components of the metabolic syndrome, a cluster of interrelated clinical features including insulin resistance, dyslipidemia, hypertension, and visceral

obesity [27]. T2DM and NAFLD are major health issues associated with the worldwide epidemic of obesity [28].Insulin resistance plays a major role in the pathogenesis of T2DM-NAFLD and is considered a key factor in initiation and perpetuation of NASH [29] Compared with non-diabetic individuals, people with type2 diabetes are at increased risk of developing non-alcoholic fatty liver disease (NAFLD), and have a higher risk of developing fibrosis and cirrhosis [30]. A strong association exists between NAFLD and type2 diabetes, with NAFLD found in up to 70% of patients with type 2 diabetes [31]. Furthermore, the presence of type 2 diabetes is associated with a more progressive course and higher rate of progression to cirrhosis [32] .Thus prediction and early intervention of dysglycemia in NAFLD might have additive benefits in reducing cardiovascular risk and decreasing the rate of NAFLD progression [33].

The pathogenesis of NAFLD was originally described by the 'two-hit hypothesis', and subsequently, modified as the 'multi hit hypothesis', which describes the first hepatic insult as the dysregulation of fatty acid metabolism, leading to steatosis. Insulin resistance plays a central role in the first insult, contributing to an imbalance between factors that promote hepatic fat accumulation (free fatty acid flux to the liver and de novo lipogenesis) and factors that prevent fatty acid build-up (fatty acid export and oxidation). This renders hepatocytes susceptible to the secondary insults ('multiple hits') of adipokine induced liver injury, oxidative and endoplasmic reticulum (ER) stresses, mitochondrial dysfunction, and hepatic apoptosis, which subsequently promote the transition from simple steatosis to NASH [34].In addition to hepatic insulin resistance, NAFLD is associated with a defect in insulin-mediated suppression of lipolysis, in keeping with insulin resistance in adipose tissues [35] .The insulin resistance might be an intrinsic defect in NAFLD, similar to that in type 2 diabetes, and that blunted insulin responsiveness at the level of the adipocytes might contribute to hepatic steatosis through excess

free fatty acid flux to the liver [36]. Isotope-tracer studies in obese humans with NAFLD on a low-fat diet showed that nearly 60% of hepatic triglycerides comes from FFA derived from adipose tissues, 26% from de novo lipogenesis and 15% from diet [37].This would suggest that, in the absence of a high-fat diet, the increased release of fatty acids from adipose tissues is the predominant source of excess hepatic fat accumulation.

2.4 Alcoholic liver disease

The liver is the main organ of alcohol metabolism. Alcohol is metabolized in the liver in three ways: (1) by the enzyme alcohol dehydrogenase (ADH); (2) by cytochrome P-4502E1 (CYP2E1); and (3) by mitochondrial catalase. Only the first two pathways are of practical significance-ADH finds use in the degradation of limited quantities of alcohol, while alcohol-induced CYP2E1 takes place in excessive alcohol intake. Apart from the liver, ADH is also present in the gastric mucosa and the assumption is that individuals with low gastric ADH activity are more susceptible to alcoholic liver disease. This may also help to explain why women who have decreased gastric ADH activity are more susceptible to developing alcoholic liver disease [38].

Both enzymes convert alcohol to acetaldehyde, which is in part responsible for the liver injury too. However, the process of liver injury is much more complex resulting from biochemical, genetic, cellular, immunological and humoral disorders in connection with the intake and metabolism of excessive quantities of alcohol. A major role is played there by oxidative stress (which is mainly due to alcohol-induced CYP2E1), by simultaneous shortage of antioxidants in the hepatocytes [38].

Alcoholic fatty liver is characterized by lipid droplet accumulation in the cytoplasm of hepatocytes, and is one of the earliest pathologic alterations in the liver. Accumulation of lipids in the hepatocytes makes the liver susceptible to inflammatory mediators or other toxic agents, leading to further progression to hepatitis and eventually to fibrosis. Alcohol consumption may affect multiple pathways of hepatic lipid metabolism including de novo lipogenesis, fatty acid oxidation, lipid uptake, and lipid export in the form of very low density lipoproteins [39].

However, recent studies have suggested that extra hepatic factors such as adiponectin critically modulate hepatic lipid metabolism [40]. White adipose tissue (WAT) is a major organ for body fat storage, and also functions as an endocrine organ. The hormones secreted by WAT are adipokines, and two of the most important adipokines related to energy homeostasis are adiponectin and leptin. Both adiponectin and leptin critically modulate hepatic lipid homeostasis toward reduction of lipid content in the liver [41]. Leptin critically regulates whole-body energy homeostasis by inhibiting energy intake and stimulating energy expenditure [42]. Lacking functional leptin develop not only obesity but also fatty liver. Therefore, adipose tissues via adipokine secretion significantly affect lipid homeostasis in the liver. Alcohol exposure has been shown to affect adipose mass and adipokine secretion in both humans and animals. Patients with alcoholism have lower body mass index (BMI) and fat mass (FM) but higher liver fat levels [43]. Studies in humans have also found that the serum leptin concentration was reduced by either chronic alcohol consumption or acute alcohol abuse. Chronic alcohol exposure in rodents reduced adipose tissue weight, and serum adiponectin and leptin, concentrations in association with the development of fatty liver. Administration of exogenous adiponectin or stimulation of endogenous adiponectin production attenuated alcoholic fatty liver [44].

Patients with alcoholic liver disease have a high relative risk of suffering diabetes. This risk is directly related to the amount of ingested alcohol, as it rises 2-fold in patients ingesting more than 270 g of alcohol per week compared with those ingesting less than 120 g/wk. Acute alcohol ingestion produces a significant reduction in insulin-mediated glucose uptake. On the other hand, patients with chronic alcoholism frequently have chronic pancreatic damage and injury of pancreatic islet β-cells resulting in DM [45].

Excess alcohol consumption is common and could coexist with NAFLD. Merely focusing on NAFLD by excluding subjects using excessive amounts of alcohol may thus underestimate the prevalence of the MetS and type 2 diabetes in subjects with elevated LFTs. This would seem particularly important as the long-term prognosis both with respect to the development of cirrhosis and total mortality is much worse for patients with AFLD than with NAFLD [46].

Cirrhosis does not develop below a lifetime alcohol consumption of 100 kg of undiluted alcohol. This amount corresponds to an average daily intake of 30 grams of undiluted alcohol for 10 years. Heavy alcoholics consuming at least 80 g of alcohol per day for more than 10 years will develop liver disease at a rate of nearly 100%. A detailed study of 256 heavy drinkers admitted to hospital not because of liver complaints, found steatosis at a rate of 45%, steatohepatitis at 34%, steatohepatitis with cirrhosis at 10% and cirrhosis alone at 10% in their liver biopsies [47].

2.5 Liver and diabetes

Type 2 diabetes (T2D) characterized by hyperglycemia and dyslipidemia caused by islet ß-cells being unable to secrete adequate insulin in response to varying degrees of longstanding insulin resistance (IR) in genetically predisposed individuals poses an enormous burden on modern societies owing to its worldwide explosion, the multi-organ damage and its direct and indirect

costs [48]. In recent years, the topic "Hepatogenous diabetes" – a definition coined in 1906 to describe the high incidence of diabetes in cirrhotic patients has gained renewed interest. Clinical observations support that impaired life expectancy of patients with T2D is not only linked to vascular complications and end-stage renal disease but is also associated with cirrhosis and hepatocellular carcinoma (HCC) [49]. Moreover, insight that recently, much emphasis is being placed on non-alcoholic fatty liver disease (NAFLD), the most common liver disorder in many Western countries and an important chronic liver disease in Asia [50].

2.6 Hepatogenous diabetes

An association between diabetes mellitus and liver cirrhosis was first described by Bohan in 1947 and named as hepatogenous diabetes, in which 57% of cirrhotic patients showed increased insulin resistance. Various pathogenetic factors are involved in development of the insulin resistance. Serum insulin levels are higher in diabetic patients with chronic liver disease than those in patients with lifestyle-related DM, suggesting that besides over-eating, obesity and physical inactivity, distinctive factors may underlie the pathophysiology of hyperinsulinemia in patients with chronic liver disease. Since blood glucose is delivered to the liver through the portal vein, hyper insulinemia in patients with liver cirrhosis may be secondary to either hepatic parenchymal cell damage or to portal-systemic shunting. The rate at which insulin is degraded in the liver is reduced in patients with liver cirrhosis [51].

The prevalence of diabetes mellitus in patients with liver cirrhosis is higher compared to that in the general population. The prevalence of diabetes has been reported to be between 30% and 70% depending on the etiology [52].

Cirrhosis may contribute to the development of T2DM through numerous factors such as reduced insulin clearance with peripheral hyperinsulinemia, which could contribute to the development of insulin resistance through the down-regulation of insulin receptors. Nevertheless, interaction of the cause of liver cirrhosis with environmental factors may also play a significant role in the link between cirrhosis and diabetes rather than development of cirrhosis alone [53].

About 20-40% chronic liver disease will progress into cirrhosis in approximately 15 years later. Patient with cirrhosis can suffer resistance of insulin, from impaired glucose tolerance to diabetes mellitus [54].

2.7 Viral hepatitis

Hepatitis C virus (HCV) infection is an important public health problem which currently affects more than 170 million people (about 3% of world population) out of which 55-80% have chronic infection. A meta-analysis showed that HCV increases the risk of T2DM by 1.8 times in excess of that posed by relative degree of liver pathology. The link between the HCV and diabetes was first reported by Allison *et al*., in 1994 and later explored by Simo and colleagues in 1996 cited by Ahmed G & Adam A [55].

The initial idea that patients with T2DM have more parenteral exposures because of use of finger stick devices and thus are at an increased risk of contacting blood borne infections such as HCV was disproved by a study from France in 1998 [56]. A large retrospective survey of 1332 Italian patients with cirrhosis found that type 2 diabetes mellitus was present in 23.6% of those with HCV infection and in 9.4% of those with HBV infection. In addition, type 2 diabetes mellitus was closely correlated with age and severity of cirrhosis. In a similar large U.S. study in 1117 patients with chronic viral hepatitis, the prevalence of type 2 diabetes mellitus was higher in

those with HCV-related disease than in those with HBV-related disease (21% vs.12%, respectively) [55].

Recent studies have suggested that HCV infection is associated with an increased risk of development of T2DM, and that T2DM is more common among patients with chronic HCV infection than in patients with other liver diseases or in the general population, irrespective of whether or not hepatic cirrhosis is present [55].

A community-based cohort survey performed in southern Taiwan enrolled 4958 persons aged = 40 years without T2DM. After a follow-up of 7 years, 474 cases of incident T2DM were recorded: overall, 14.3% of anti-HCV positive, 7.5% of HBsAg positive, and 8.6% of sero negative individuals developed T2DM during the study. Compared to anti-HCV negative individuals, anti-HCV positive persons had a higher cumulative incidence of T2DM ($P< 0.0001$) [57]. A case control study was performed in Ethiopia at Jimma University Specialized Hospital on a total of 604 study subjects. From patient's serum sample, HCVAb screening was done by rapid antibody screening test and the prevalence of HCV in type II diabetes and non diabetic controls was 9.9% and 3.3%, respectively. Association of Hepatitis C Virus Infection with Type II Diabetes is reported in Ethiopia [58].

2.8 Hepatic carcinogenesis

Hepatocellular carcinoma, the most common primary liver cancer, ranks fourth among the most prevalent malignancies worldwide and third leading cause of cancer-related deaths [59]. For primary liver cancer, most of which (> 90%) comprises hepatocellular carcinoma, sufficient evidence already exists for a positive association with diabetes mellitus, as illustrated by several meta-analyses showing 2–4-fold increase of summary relative risk (RR) in diabetic vs. non-

diabetic individuals [60]. Several mechanisms could favor the development of HCC in the setting of NAFLD, including abnormal glucose metabolism, hepatocyte iron deposition, age and advanced fibrosis. The subclinical inflammatory state associated with, steatosis, oxidative stress and unbalanced adipocytokine ratio (i.e. increased IL-6, leptin TNF-α and decreased adiponectin) could all play a major role in cell growth kinetics and promotion of DNA damage all of which provide a favorable environment for the development of HCC [61].

The phosphoinositide 3-kinase (PI3K)/phosphatase and tensin homolog (PTEN)/Akt axis is a key regulator of crucial cellular functions such as insulin and other growth factor signaling, glycolipidic homeostasis, cell survival and apoptosis [62]. Not only is PTEN a tumor suppressor but, interestingly, it is dysregulated in obesity, and T2DM, therefore representing an ideal metabolic pathway accounting for the development of HCC in the setting of metabolic disorders such as T2DM and NAFLD [63]. Interestingly, recent studies suggest that the type of anti diabetic drug treatment used may modulate the risk of developing HCC, by increasing insulin level and decreasing insulin sensitizers [64].

2.9 Management of type 2 Diabetic mellitus

Current recommendations for the management of patients with type 2 diabetes include lifestyle interventions such as diet, physical activity, weight loss and smoking cessation are an integral part of any diabetes management plan [65]. Type 2 diabetes mellitus (T2DM) is a progressive disease characterized by raised glucose levels which is associated with macrovascular and microvascular complications as well as dyslipidaemia, that increase morbidity and mortality. There are various medications introduced to reduce glucose levels and improve DM patients'

clinical status, pharmacological therapy will be needed to reach treatment goals in many patients [66]. Some of them are:-

2.9.1 Metformin

Metformin belongs to the class of biguanides, which were introduced into diabetes treatment over 40 years ago. Metformin improves diabetes control by reducing insulin resistance, mainly in the liver but also in the skeletal muscle, without increasing pancreatic beta-cell secretion. The reduction in hepatic glucose production by metformin may be explained at least in part by the lower rate of lipid oxidation [67].

Metformin is now established as a first-line anti-diabetic therapy for the management of type 2 diabetes [68]. Metformin ameliorates the insulin resistance (IR) primarily in liver, muscle, and adipose tissue by reducing the hepatic glucose output, largely due to a reduction in the rate of gluconeogenesis and glycogenolysis. The beneficial cardiovascular effects of metformin have been observed in the UK Prospective Diabetes Study (UKPDS). According to the results of this study, patients who received metformin benefited from clinically and statistically significant improvements in the risk of all-cause death, diabetes related death, myocardial infarction, and reduction of diabetes related complications. Also in UKPDS it has been shown that metformin provides protection from macro vascular diabetic complication independently of glycaemic lowering effects. Regarding weight loss in obese diabetic subjects and proteinuria, concomitant and simultaneous treatment of diabetic dyslipidemia and treatment with metformin have all been shown to reduce urinary albumin excretion and ultimately reduction in microvascular and macrovascular complications independent of tight glycaemic control [69].

The most common side-effects that are reported with metformin use are gastrointestinal, including a metallic taste, nausea, vomiting, diarrhoea, and abdominal discomfort. A very rare, but serious, adverse effect that occurs with metformin, is lactic acidosis. Patients with renal impairment, concurrent liver disease, or those who abuse alcohol, have a past history of lactic acidosis, and decreased tissue perfusion due to infection, are at increased risk of lactic acidosis. Therefore, metformin doses should be reduced in patients with renal impairment, and treatment may need to be discontinued during infection, i.e. influenza, upper-respiratory tract infection, or urinary tract infection. Metformin should be stopped before radiological procedures involving iodinated contrast material, to prevent lactic acidosis in the event of contrast-induced renal failure, and may be restarted once renal function is restored. Doses usually start at 500 mg, with an upper limit of 2550 mg per day (850 mg three times daily) [70].

2.9.2 Sulfonylureas

Sulfonylurea (SU) drugs have been the mainstay of anti-diabetic therapy for many years. Their primary mechanism of action is enhancement of insulin secretion. They initiate their action by binding to a specific sulfonylurea receptor on pancreatic beta-cells. This closes a potassium-dependent adenosine triphosphate channel, leading to decreased potassium influx and depolarization of the beta-cell membrane. This results in increased calcium flux into the beta-cell, activating a cytoskeletal system that causes translocation of secretory granules to the cell surface and extrusion of insulin through hexocytosis . Insulin released by the pancreas enters the portal vein, and the resultant portal hyperinsulinemia suppresses the elevated basal rate of hepatic glucose production [72].

Understandably, in the face of SU therapy, circulating insulin concentrations are increased. As a result, and despite the presence of insulin resistance, glucose concentrations fall. The possibility that such agents may also directly enhance peripheral glucose disposal (i.e., decrease insulin resistance) has also been raised. However, the peripheral effects of SUs are most likely secondary to a reduction in glucotoxicity [73].

Sulfonylureas remain largely used in the management of T2DM and are currently positioned as second-line after failure of metformin monotherapy. They are associated with a higher risk of severe hypoglycemia, compared with metformin and more recent glucose-lowering therapies, especially in the elderly population and in patients with renal or liver disease. Among the possible extra-pancreatic effects of sulfonylureas, a reduction of the hepatic extraction of insulin has been reported, which could contribute to increase peripheral insulin concentrations [74].

Sulfonylureas are effective at increasing insulin secretion if the patient has functional pancreatic beta-cells, but this can also cause hypoglycaemia and weight-gain. Sulfonylureas are also contra-indicated in patients with ketoacidosis and should be avoided in patients with acute porphyria. There are currently three fully-subsidised sulfonylureas in New Zealand, glipizide, gliclazide and glibenclamide. Glipizide and gliclazide are shorter-acting and are preferred, with caution, in older patients. Glibenclamide is long-acting and should be avoided in older patients [75].

The combination of metformin and sulfonylurea (SU) is one of the most commonly used and can attain a greater reduction in HbA1c (0.8–1.5%) than either drug alone. Epidemiological investigations suggest that patients on SUs have a higher cardiovascular disease event rate than those on metformin. Patients who started SUs first and added metformin also had higher rates of cardiovascular disease events compared with those who started metformin first and added SUs. These investigations are potentially affected by unmeasured confounding variables [71].

2.9.3 Insulin

Insulin is eventually required for many people with type 2 diabetes and early initiation can be appropriate. Beta-cell function declines linearly and after ten years 50% of people with type 2 diabetes will require insulin. Insulin has a greater blood glucose lowering ability than any other hypoglycaemic medicine, and early initiation may reduce beta-cell damage and is thought to slow disease progression. Early initiation of insulin should be strongly considered for people with type 2 diabetes who have significant hyperglycaemia, e.g.HbA1c> 65 mmol/mol, particularly if there are signs such as ketonuria and weight loss. If there are immediate health concerns, insulin initiation, even if temporary, may be the only treatment option [75].

Insulin as added to metformin -based regimens has been shown to improve glycemic control, limit changes in body weight, reduce hypoglycemia incidence, and to reduce insulin requirements (sparing effect), allowing a 15–25% reduction in total insulin dosage [71].

2.10 Significance of the study

Diabetic Mellitus is a common incurable chronic disease. During the past decade, DM has emerged as an important clinical and public health problem throughout the world. The prevalence of diabetes mellitus is reaching epidemic proportions, in large part because of obesity and sedentary life style in both adults and children. Likewise in Ethiopia, diabetes has become more common and it is a fast growing disease.

The prevalence of liver diseases is higher in patients who have suffered from type 2 diabetes mellitus (T2DM). Diagnostic and early therapeutic interventions are needed for treating liver disease in patients at risk for developing T2DM. On the other hand, prevention or early diagnosis of progressive liver disease is needed in T2DM patients. For that reason, the present study is

attempting to provide a comparative explanation of liver function tests in type 2 diabetic patients who are receiving anti- diabetic drugs by using lipid profile and enzyme studies in patients visiting Tikur Anbessa Specialized Hospital. Therefore, this study helps T2DM patients receiving different anti-diabetic drugs, to know the status of their liver function and if they have liver function abnormality, and to treat them earlier. It may be useful as a base line for other researchers conducting study in related topics and also for organizations working with DM. Besides, it may serve as a guide for prevention and early detection of liver problems in type 2 diabetic patients.

2.11 Hypothesis of the study

Anti-diabetic drugs are causative agents for elevation of serum liver enzyme levels and lipid profile.

3. Objective

3.1 General Objective of the study

- To investigate profile of liver function tests among type 2 diabetic patients who are receiving different anti-diabetic drugs attending Tikur Anbessa Specialized Hospital

3.2 Specific Objective

- To assess biomarker enzymes (ALT, AST, ALP) of the liver among type 2 diabetic patients who are receiving different anti-diabetic drugs.

- To estimate lipid profiles (LDL, HDL, TG) among type 2 diabetic patients who are receiving different anti-diabetic drugs.
- To assess total protein and bilirubin among type 2 diabetic patients who are receiving different anti-diabetic drugs.
- To asses fasting blood sugar among type 2 diabetic patients who are receiving different anti-diabetic drugs.
- To asses body mass index among type 2 diabetic patients who are receiving different anti-diabetic drugs.

4. Materials and methods

4.1 Study area

The study was conducted in Tikur Anbessa Specialized Hospital and control patients were recruited from Federal Referral Police Hospital, Addis Ababa; because, the patients who visited TASH were already on advanced stages of medications. Hence newer patients who were to be recruited without medications were not available. Therefore, recruitment of patients before the start of any medications was done at Federal Referral Police Hospital, Addis Ababa. The numbers of patients attending diabetic clinic of TASH during sampling period were 850 while patients visiting Federal Referral Police Hospital were 1250.

4.2 Study design and period

A hospital based cross-sectional study was conducted due to limitation of time and money in Addis Ababa, Tikur Anbessa Specialized Hospital, from January 2015 to March 2015. In this period, blood sample collection was done.

4.3 Source of population

The source of population was diabetic patients who were attending diabetic clinic at Tikure Anbessa Specialized Hospital and Federal Referral police Hospital in Addis Ababa.

4.4. Study population

The study population consisted of 70 type 2 diabetic patients who are receiving different anti-diabetic drugs and 35 diabetic patients who did not started any medication. Hence a total 105 diabetic patients were considered in the study.

4.5. Inclusion and Exclusion criteria

4.5.1. Inclusion criteria

Patients of age greater than 18 years (both sexes) who are diabetic patients receiving different anti-diabetic drugs attending Tikur Anbessa Specialized Hospital and T2DM patients from the Federal Referral Police Hospital who had not started taking any medications included as a control group in this study.

4.5.2. Exclusion criteria

Patients who had any clinical evidence of cirrhosis or other causes of chronic liver disease, diagnosed type 1 diabetic patients, pregnant and lactating mothers, children less than 18 years of

age, type 2 diabetic patients who used HIV drugs and patients drinking alcohol greater than twice a week were excluded from this study.

4.6 Sampling technique and Sample Size determination

The sample sizes were estimated by using a single proportion formula and calculated as follows.

- P= assumed the highest population proportion prevalence of diabetes mellitus in Ethiopian adults 4.36%, [5]
- 5% marginal error (d) to get sample size and
- Confidence interval (CI) of 95%.
- n == Z^2pq / d^2

  n = Sample size

  p = Proportion of DM= 0.0436

  d = Margin of error =0.05

  q = 1-p =1- 0.0436 =0.9564

  Z = 1.96 at 95% Confidence Interval (CI)

$$n == \frac{(1.96)^2 \times 0.0436 \times 0.9564}{0.05 \times 0.05} = \frac{0.160191}{0.0025} = 64.0764 = 64$$

- To avoid non response rate 10% is added. So the total sample was 70.

Simple random sampling technique was applied among type 2 diabetic patients who have been attending Tikure Anbessa specialized hospital for medication. To select patients for the study, two options were assigned (number one and number two) and Nurses who collected the sample

were instructed. The patient who had been calling number one and then volunteered to participate in the study was selected. Those patients who have been calling number one but did not volunteer to participate in the study and those calling number two were excluded. To recruit patients for control sampling, which was carried out at the Federal Referral Police Hospital, selection was made from the patients who reported at the hospital during the study period. Patients whose fasting blood glucose levels were $\geq$ 115 mg/dl were selected. 70 patients were recruited for study group and 35 patients were recruited for control group, in the ratio of 2:1.

4.7 Study variables

4.7.1 The independent variable:

- Socio-demographic characteristics (age and sex)
- Duration of the disease,
- Type of drugs
- Alcohol intake status

4.7.2 Dependent variable

- Liver function test indicated by level of AST, ALT, ALP, Bilirubin, Albumin, Total protein, HDL, LDL, TG and Cholesterol.

4.8 Data Analysis

Collected quantitative data was coded, entered to computer, processed, edited, and analyzed using EPI-INFO and SPSS (20th version) and expressed at 95% confidence interval and the p-

value were considered significant at $p < 0.05$. Then data computed using appropriate statistical methods (mean, standard deviation, p-value, F test statistic value and one-way ANOVA) and the results were presented using tables and figures. Clinical and laboratory data were expressed as the mean ± standard error of mean (SE). Differences in the means between the studies group and control group were evaluated by independent samples t-test and chi ($\chi 2$) tests.

Correlations were evaluated by the Pearson correlation test. The data collected during the current study were recorded and analyzed statistically to determine the significance of different parameters by using SPSS package for windows version 20.0.

4.9 Data Quality Assurance

The data quality starts with the sample collection. The sample had been taken in aseptic techniques and collected with considering proper procedure. The kit had been made free from contamination and kits were checked for consistency. Collected results were checked for completeness on daily basis by the immediate supervisor. Attention in data insertion to software on computer. The completed result was rechecked repeatedly to maintain the quality of data.

4.10 Ethical consideration

Ethical clearance was obtained from Research and Ethical Committee of the Department of Biochemistry, School of Medicine, College of Health Sciences, Addis Ababa University after full review was conducted meeting No. DRERC 04/14 attended by the research committee and give approval with protocol number of M.Sc. Thesis 06/14. Structured Questionnaire (attached as appendix) and consent form was prepared with detailed explanation of objectives, risks, and benefits to the study subject and the confidentiality of responses were given to participants. Data

were collected after obtaining informed consent and agreement from the patients under study. Sample collection was performed by trained health professionals following ethical steps and procedures.

4.11 Method of Data collection and Analysis

Data was collected by well trained Nurses. Data collection form was designed to record sex, age, weight, height, BMI, alcohol intake status, and medical history of each patient. Portable mechanical analog scales were used to measure height and weight, respectively.

4.11.1 Blood collection

Five ml of venous blood were drawn from each volunteer patient using a disposable plastic syringe. The blood was poured in a test tube and then centrifuged after it clotted. Serum was kept at -80°C in the refrigerator till used. AST, ALT, ALP, TB, TC, TG, HDL and FBS were measured by (Human gesellschaft for biochemica and diagnostic mbh – Germany). TP and albumin measured by (Linear chemicals S.L, Spain) according to the manufacturer's procedures.

4.11.2 Body Mass Index (BMI)

Body Mass Index is a useful clinical calculation to diagnose obesity because it is correlated with total body fat and is relatively unaffected by height. It is most often used to diagnose obesity, but it is equally applicable to defining those who are underweight. There are some limitations to the BMI since it will overestimate body fat in persons who are very muscular and underestimate body fat in persons who have lost muscle mass, such as the elderly [76].

BMI= weight (Kg) / Height^2 (meter^2)

Table 1:- **Classification of Overweight and Obesity by BMI.**

BMI (kg/M^2)		Obesity class	Increase disease risk relative to normal Weight and waist circumference
Underweight	< 18.5		-
Normal	18.5-24.9		-
Overweight	25.0-29.9		Increased
+Obesity	30.0-34.9	I	High
	35.0-39. 9	II	Very High
Extreme Obesity	≥ 40	III	Extremely High

4.12 Laboratory Testing Methods

4.12.1 Serum HDL determination methods

The very low and the low density lipoproteins from serum are precipitated by phosphotungstate in the presence of magnesium chloride [77]. After removal by centrifugation the clear supernatant is used for the determination of HDL-cholesterol.

Principles of the Method

The basic principle of the method is as follows. The apoB containing lipoproteins in the specimen react with antibodies to apoB that renders them nonreactive with the enzymatic cholesterol reagent under conditions of the assay. The enzymes used are also pegylated, and this allows them to react only with HDL and not with antibody-bound LDL, VLDL or chylomicrons. The apoB containing lipoproteins are thus effectively excluded from the assay and only HDL is detected under the assay conditions.

The HDL-Cholesterol test is a two reagent homogenous system for the selective measurement of serum or plasma HDL-Cholesterol in the presence of other lipoprotein particles. The assay is comprised of two distinct phases. In faze one; it is likely that in the presence of slightly alkaline buffer and magnesium sulfate and dextran sulfate selectively form water-soluble complexes with LDL, VLDL, and chylomicrons, which are resistant to PEG-modified enzymes. In phase two the cholesterol concentration of HDL cholesterol is determined enzymatically by cholesterol esterase and cholesterol oxidase coupled with PEG to the amino groups (approx. 40%).

$$\text{HDL-cholesterol esters} + H_2O \xrightarrow[\text{esterase}]{\text{PEG cholesterol}} \text{cholesterol} + \text{free fatty acids}$$

Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by cholesterol esterase.

$$\text{cholesterol} + O_2 \xrightarrow[\text{oxidase}]{\text{PEG cholesterol}} \text{cholestenone} + H_2O_2$$

In the presence of oxygen, cholesterolis oxidized by cholesterol oxidase Δ^4 cholestenone and hydrogen peroxide.

$$2\,H_2O_2 + \text{4-amino-antipyrine} + HSDA^{*} + H^{+} + H_2O \xrightarrow{\text{peroxidase}} \text{Purple-blue pigment} + 5H_2O$$

HSDA= N-(2-hydroxy-3-sulfopropyl)-3, 5-dimethoxyaniline

In the presence of peroxidase, the hydrogen peroxide generated reacts with 4-aminoantipyrine and HSDA to form a purple-blue dye. The color intensity of this dye is proportional to the cholesterol concentration and can be measured spectrophotometrically.

Procedure

Ten micro liter (10µL) serum samples were added into the sample cups and put on the sample disk which rotates to bring the desire sample cup into position next to the sample probe for specimen sampling. 1000µL buffer and 1000µL substrate were pipetted into reagent bottles leveled for HDL-C and put on the reagent disk. Then on the screen menu of the machine HDL-C was entered as a parameter to be tested. The sample probe was pipetted sample from the sample disk and transferred to the reaction disk which contains cuvettes. On the other side of the machine, the reagent probe was pipetted reagents from the reagent disk and transferred it into rotatable reaction disk holding reusable cuvettes with a stirring paddle to stir or mix thoroughly the sample and the reagents. The cuvettes were immersed in to reaction water bath and incubated at 37^0C for 5 minutes. Next the reaction disk was rotated the cells to all reaction stations including the photometer light path. Finally, the light was passed through the cuvettes and absorbance of the sample was measured at 500nm ((Human gesellschaft for biochemica and diagnostic mbh – Germany)

4.12.2 Colorimetric determination of total cholesterol

Total cholesterol was measured enzymatically in serum in a series of coupled reactions that hydrolyze cholesteryl esters and oxidize the 3-OH group of cholesterol. Cholesterol esters are hydrolyzed to free cholesterol by cholesterol ester hydrolase (CE). The free cholesterol produced is oxidized by cholesterol oxidase (CO) to cholest-4-en-3-on with the simultaneous production of hydrogen peroxide, which oxidatively couples with 4-aminoantipyrine and phenol in the presence of peroxidase to yield Quinoneimine dye with maximum absorbtion between 500-550 nm.

Reactions

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{Cholesterol ester hydrolase}} \text{Cholesterol} + \text{Fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholest-4-ene-3-one} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{phenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4H_2O$$

The test comes in the form of a commercial kit in which serum sample is incubated with enzymes and reagents from the kit and the change in absorption at 500nm is measured spectrophotometrically. This change in absorption is proportional to the concentration of total cholesterol in the serum sample and can be calculated by comparison with absorption changes that occur with standard solutions containing known cholesterol Concentrations.

Procedure

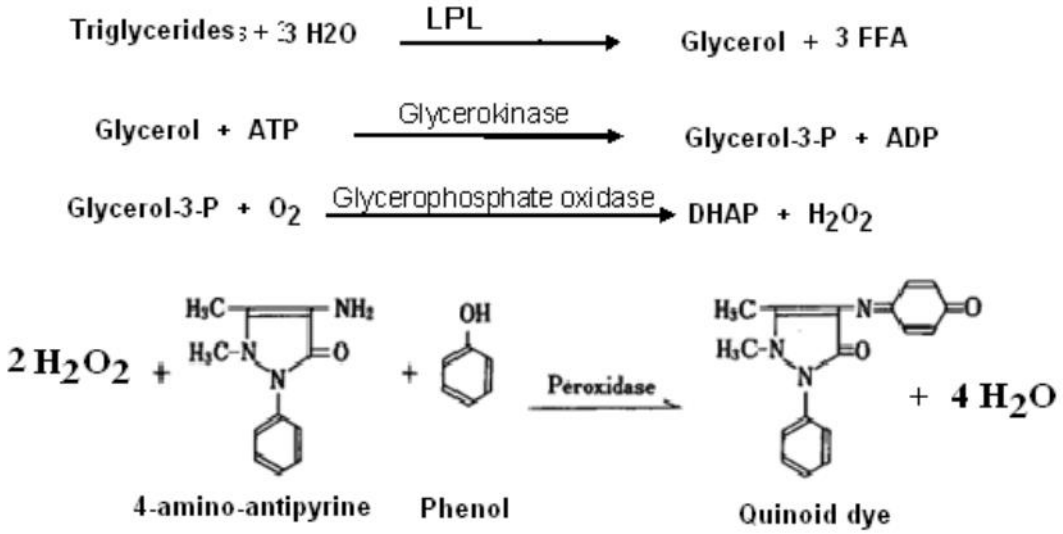
Ten microliter (10μL) serum sample were added into the sample cups and put on the sample disk which rotates to bring the desire sample cup in to position next to the sample probe

for specimen sampling. 1000µL reaction reagent (4-Aminophenazone, phenol, peroxidase, cholesterol esterase, cholesterol oxidase) were pipetted into reagent bottles leveled for TC and put on reagent disk and then on the screen menu of the machine TC was entered as a parameter to be tested. The sample probe was pipetted sample from the sample disk and transferred to the reaction disk which contains cuvettes. On the other side of the machine, the reagent probe was pipetted reagents from the reagent disk and transferred it into reaction disk which is a large rotatable disk holding reusable cuvettes with a stirring paddle to stir or mix thoroughly the sample and the reagents. The cuvettes were immersed into reaction water bath and incubated at 37^0C for 5 minutes. Next the reaction disk was rotated the cells to all reaction stations including the photometer light path. Finally, the light was passed through the cuvettes and absorbance of the sample was measured at 500nm ((Human gesellschaft for biochemica and diagnostic mbh – Germany)

4.12.3 Serum Triacylglycerol assay method

The method is based on the enzymatic hydrolysis of triglycerides to glycerol and free fatty acids (FFA) by lipoprotein lipase (LPL). Glycerol is converted to glycerol-3-phosphate (G3P) and adenosine-5-phosphate (ADP) by glycerol kinase and ATP. G-3-P is oxidized by glycerol phosphate oxidase to form dihydroxy acetone phosphate (DHAP) and hydrogen peroxide (H_2O_2). In the presence of peroxidase and H_2O_2, 4-aminoantipyrine couples with phenol to form a coloured product (a quininoid dye) that can be measured spectrophotometrically at a wave lengthof 500nm.

Reactions Principle:



ESPAS: N-ethyl-N-sulfopropyl-m-anizidine

The triglyceride test comes in the form of a commercial kit containing the reagents, reactants and enzymes needed. Serum samples will be incubated with the kit reagents and enzymes for 5 minutes at 37^0C and absorbance measured at 500 nm against the reagent blank and against known concentrations of standard triglyceride concentrations. The change in absorbance is proportional to the concentration of triglyceride in the serum sample.

.**Procedure**

Ten micro liter (10µL) serum samples were added into the sample cups and put on the sample disk which rotates to bring the desire sample cup into position next to the sample probe for specimen sampling. 1000µL buffer and 1000µL substrate were pipetted into reagent bottles leveled for TG and put on the reagent disk. Then on the screen menu of the machine TG was entered as a parameter to be tested. The sample probe was pipetted sample from the sample disk and transferred to the reaction disk which contains cuvettes. On the other side of the machine, the reagent probe was pipetted reagents from the reagent disk and transferred it

into rotatable reaction disk holding reusable cuvettes with a stirring paddle to stir or mix thoroughly the sample and the reagents. The cuvettes were immersed in to reaction water bath and incubated at 37^0C for 5 minutes. Next the reaction disk was rotated the cells to all reaction stations including the photometer light path. Finally, the light was passed through the cuvettes and absorbance of the sample measured at 500nm (Human gesellschaft for biochemica and diagnostic mbh – Germany)

4.12.4 LDL-cholesterol

Most of the circulating cholesterol is found in three major lipoprotein fractions: very low density lipoproteins (VLDL), LDL and HDL.LDL-cholesterol is calculated from measured values of total cholesterol, triglycerides and HDL cholesterol according to the Friedewald equation:

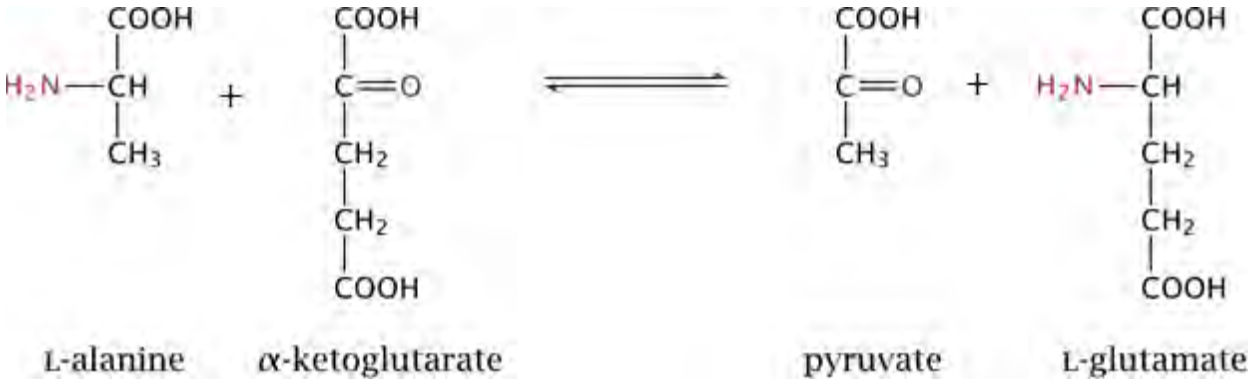
[LDL C] = [Total Cholesterol] - [HDL] - [TG]/5

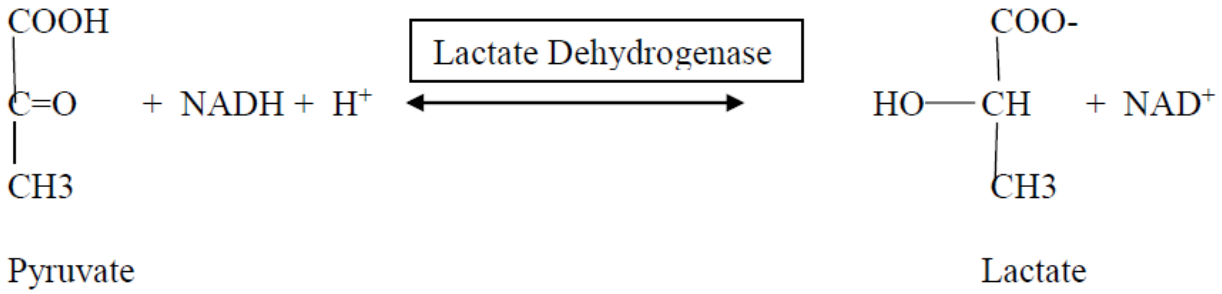
Where [TG]/5 is an estimate of VLDL-C and all values are expressed in mg/dL. The equation is derived from another equation, [Total Cholesterol] = [VLDL-C] + [LDL-C] + [HDL-C], but TG are easier to estimate than VLDL and [TG/5] is a good estimate of VLDL, although the Friedewald equation is not valid for calculating LDL if the serum TG is above 400 mg/dL

4.12.5 Alanine Aminotransferase (ALT) assay method

ALT catalyzes the transfer of the amino group from alanine to α-ketoglutarate with the formation of glutamate and pyruvate. The latter is reduced to lactate by lactate dehydrogenase (LDH) in the presence of reduced nicotinamide adenine dinucleotide (NADH). The reaction is monitored kinetically at 340 nm by the rate of decrease in absorbance resulting from the oxidation of

NADH to NAD+ through the activity of ALT present in the sample. The method is linear up to 800 U/L.





Reagent Composition and preparation

Reagent A: Tris 150 mmol/L, L-alanine 750 mmol/L, lactate dehydrogenase > 1350 U/L, pH 7.3; Reagent B: NADH 1.9 mmol/L, 2-oxoglutarate 75 mmol/L, sodium hydroxide 148 mmol/L, sodium azide 9.5 g/L. Mix 4 mL of reagent A to 1 mL of reagent B.

Procedure

Samples and reagents were brought to room temperature and vortexed prior to the analysis. After running normal and pathological high control, 40 samples at a time were placed in a fully automated clinical chemistry analyzer. The analyzer was programmed for appropriate wavelength, temperature and volume of plasma sample (50 µL) and 1.0 mL of reagents. The analyzer takes appropriate volume of reagent and sample, mix and incubates at 37 ^{o}C for one

minute. Based on the intensity of color compound formed, the automated analyzer reads the absorbance at 340 nm kinetically at one, two, three minutes and determines the concentration (activity) of ALT in the sample by calculating the mean of the results to obtain the average change in absorbance per minute (ΔA/min) (Human gesellschaft for biochemica and diagnostic mbh – Germany)

Calculations: The absorbance change for samples was determined as follows:

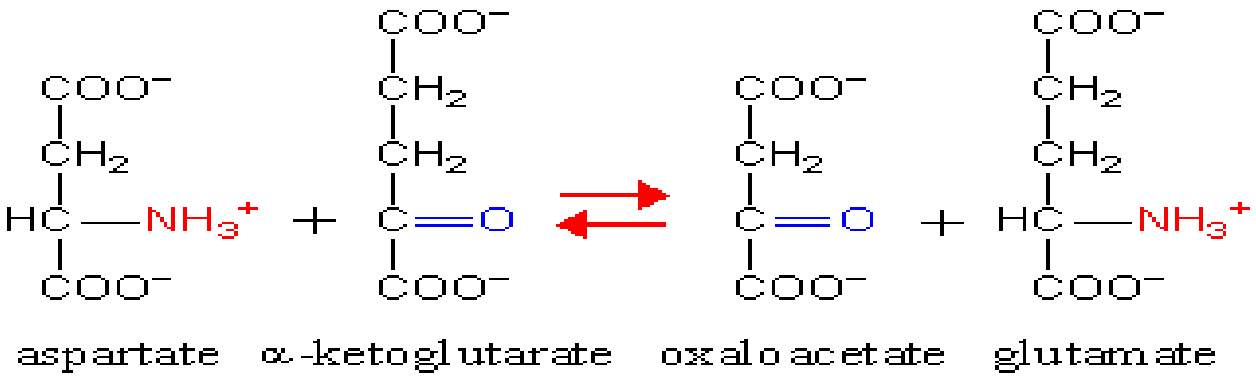
ΔA = (A2 - A1) + (A3 - A2)

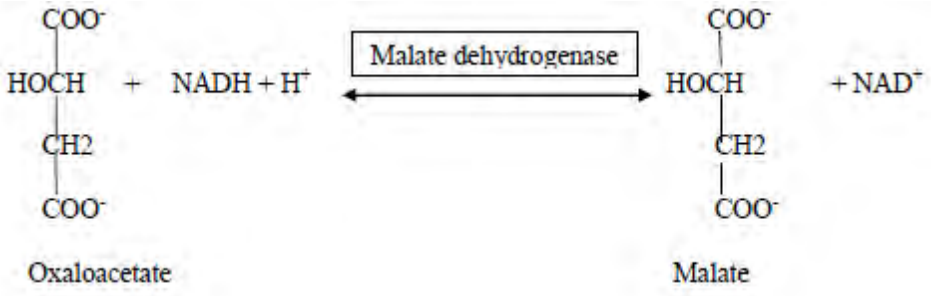
(2 - 1) + (3 - 2) min

U/L = ΔA/min x Factor

4.12.6 Aspartate Aminotransferase (AST) assay method

AST catalyzes the transfer of the amino group from asparate to α-ketoglutarate with the formation of glutamate and oxaloacetate. The latter is reduced to malate by malate dehydrogenase (MDH) in the presence of reduced nicotinamide adenine dinucleotide (NADH). The reaction is monitored kinetically at 340 nm by the rate of decrease in absorbance resulting from the oxidation of NADH to NAD+ proportional to the activity of AST present in the sample. The method is linear up to 800 U/L.





Reagent Composition and preparation

Reagent A: Tris 121 mmol/L, L-aspartate 362 mmol/L, malate dehydrogenase > 460 U/L, lactate dehydrogenase > 660 U/L, sodium hydroxide 255 mmol/L, pH 7.8; Reagent B: NADH 1.9 mmol/L, 2-oxoglutarate 75 mmol/L, sodium hydroxide 148 mmol/L, sodium azide 9.5 g/L. Mix 4 mL of reagent A to 1 mL of reagent B.

Procedure

Samples and reagents were brought to room temperature and vortexed prior to analysis. After running normal and pathological high control, 40 samples at a time were placed in a fully automated clinical chemistry analyzer. The analyzer was programmed for appropriate wavelength, temperature and volume of plasma sample (50 µL) and 1.0 mL of reagents. The analyzer takes appropriate volume of reagent and sample, mix and incubates at 37 ^{o}C for one minute. Based on the intensity of color compound formed, the automated analyzer reads the absorbance at 340 nm kinetically at one, two, three minutes and determines the concentration (activity) of AST in the sample by calculating the mean of the results to obtain the average change in absorbance per minute (ΔA/min) (Human gesellschaft for biochemica and diagnostic mbh – Germany)

Calculations: The absorbance change for samples was determined as follows:

$\Delta A = (A2 - A1) + (A3 - A2)$

(2 - 1) + (3 - 2) min

U/L = ΔA/min x Factor,

4.12.7 Total bilirubin determination method

Bilirubin is converted to colored azobilirubin by diazotized sulfanilic acid and its absorbance is measured spectrophotometrically at 540 nm. The intensity of color formed is directly proportional to the concentration of bilirubin in the specimen. Of the two bilirubin fractions in plasma–bilirubin-glucuronide and free bilirubin which is bound to albumin– only the former reacts directly, while free bilirubin reacts after being displaced from protein by an accelerator. The method is linear up to 20 mg/dL.

Total bilirubin + Sodium Nitrite + Diazotized SulfanilicAcid Azobilirubin

Reagent Composition and Preparation

RT:Sulfanilic acid 29 mmol/L, hydrochloric acid 0.24 mol/L, duposol 3% (w/v); RN: Sodium nitrite 11.6 mmol/L. Mix 1 mL RN + 4 mL RT.

Procedure

Samples and reagents were brought to room temperature and each plasma was vortexed. Into each of respective tubes the following reagents were pipetted. The reaction mixture was mixed and allowed to stand at room temperature for 2 minutes.

The absorbance of sample and standard was read against reagent blank at 540 nm using Cobas integra automated clinical chemistry analyser (Human gesellschaft for biochemica and diagnostic mbh – Germany).

Calculation: The absorbance change of standard and samples was determined as follows:

ΔA sample = A sample – A blank

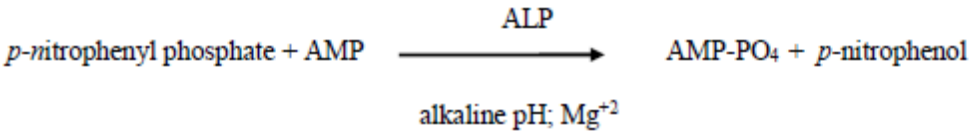
$$\Delta A \text{ standard} = A \text{ standard} - A \text{ blank}$$

Sample total bilirubin concentration was calculated using the following equation:

$$\text{Sample T. Bilirubin Concentration (mg/dL)} = \frac{\Delta A \text{ sample X}}{\Delta A \text{ standard}} \text{ (Concentration of Standard)}$$

4.12.8 Alkaline phosphates assay method

Alkaline phosphatase hydrolyses *p*-nitrophenyl phosphate and the phosphate is transferred to AMP (2-Amino-2-methyl-1-propanol.) The increase in absorbance at 405 nm at 37 0C is measured and this is proportional to the amount of alkaline phosphatase that is present in the sample. The method is linear up to 1200 U/L (Human gesellschaft for biochemica and diagnostic mbh – Germany)



Reagent Composition and Preparation

R1: Buffer: 2-Amino-2-methyl-1-propanol (AMP) and magnesium acetate; R2: Substrate: *p*-nitrophenyl phosphate 60 mmol/L, sodium azide 0.095 %. Two mL of R2 was added to one bottle of R1 and mixed thoroughly prior to use.

Procedure

After bringing samples and reagents to the room temperature, 20µL of plasma was mixed with 1.0 mL of working reagent and incubated at 37 ^{o}C for one minute. The initial absorbance was read and subsequently exactly after 1, 2 and 3 minutes.

Calculation: From the readings, automatic chemistry analyzer calculates the mean absorbance change per minute (ΔA/min).

$\Delta A/\ min = (A2 - A1) + (A3 - A2)$

(2 - 1) + (3 - 2) min

Alkaline phosphatase activity in the sample was calculated using the following factor:

U/L: ($\Delta A/min$) x Factor

4.12.9 Total protein determination method

The procedure is based on two chemical reactions. The first is the biuret reaction, in which the alkaline cupric tartrate reagent complexes with the peptide bonds of the protein. This is followed by the reduction of the Folin & Ciocalteu's phenol reagent, which yields a purple color. Absorbance of the colored solution is read at a suitable wavelength between 500 nm and 800 nm. The protein concentration is determined from a calibration curve.

Procedure

Standard Tubes was prepared by diluting the 400µg/ml Protein Standard Solution in water to a volume of 1.0 ml in appropriately labeled test tubes. 20µL of the sample was added to the appropriately labeled test tube and dilute to 1.0 ml with water. 1.0 ml of the Lowry Reagent Solution was added to Standard, Blank, and Sample tubes and mixed well, and solutions were allowed to stand at room temperature for 20 minutes. With rapid and immediate mixing, 0.5 ml of the Folin & Ciocalteu's Phenol Reagent Working Solution was added to each tube and allowed color to develop for 30 minutes. Then, the solution was transferred to cuvets and the absorbance of the Standards and Sample tubes versus the Blank at a wavelength between 500 and 800 nm will be measured. The absorbance readings were completed within 30 minutes. The absorbance values of the Standards versus their corresponding protein concentrations will be

plotted to prepare a calibration curve which will be used to determine the total protein concentration of the Sample tube (Linear Chemicals. S.L. Spain).

4.12.10 Albumin determination: Bromocresol Green (BCG) Methods

BCG is an indicator, which is yellow between pH 3.5 - 4.3. When bind to albumin the color of the indicator changes from yellow to blue- green. The absorbance of the color produced was measured in a spectrophotometer at 632nm wave lengths.

Albumin + BCG pH 4.3 Albumin-BCG complex(blue –green) (Linear Chemicals. S.L. Spain).

Procedure

4.0ml working dye solution was pipetted into test tubes then 20µl of standard, control, test sample were added separately for each measurement, mixed properly and absorbance was measured immediately within 30 seconds at 632nm after setting the instrument to zero absorbance with the working dye solution.

4.12.11 Fasting blood glucose (FBG) Determination Principle

A Senso card glucometer, which measures blood glucose levels accurately up to 600 mg/mL and is based on the glucose oxidase method, was used. Glucose reacts with oxygen in the presence of the enzyme, glucose oxidase, which oxidizes glucose to gluconolactone and this enzyme is temporarily reduced by electrons transferred from glucose (2 electrons per glucose molecule). The reduced glucose oxidase enzyme next reacts with an oxidized mediator, transferring electrons to an electrical system that creates and electrical current. The measured current is directly proportional to the concentration of glucose in the sample, which is displayed digitally on the glucometer (Human gesellschaft for biochemica and diagnostic mbh – Germany).

.5.0 Result

A total of 70 patients diagnosed with type 2 diabetic mellitus and 35 diabetic individuals who do not receive any medication as control group were selected to perform this study. Among a total of 70 diagnosed patients, 30 (42.9%) were male and 40 (57.1%) were females. The number of male were 19 (54.3%) and females were 16 (45.7%) in control group. The average age of diabetic patients on medication was 55.10 ± 10.227 years, ranging between 34 and 76 years. The average age of diabetic patients who do not receive any medication was 52.17 ± 11.8 years, ranging from 28 to 78. For diabetic patients on medication, the mean duration of diabetes was 12.3 ± 8.0, ranging from 1 to 40 years. Body mass index (BMI) was <18 kg/m^2; in 1 patients (1.4%); 26 patients (37.1%) had a BMI between 18 and 25 kg/m^2; 30 patients (42.9%) had a BMI between 25 and 30 kg/m^2; 13 patients (18.6%) with BMI>30 kg/m^2. For diabetic who don't receive any medication controls, 1(2.9%) had BMI <18 kg/m^2, 15(42.9%) had a BMI between 18 and 25 kg/m^2, 15 (42.9%) had a BMI between 25 and 30 kg/m^2, and 4 (11.4%) had a BMI>30 kg/m^2. 43 (61.5%) of patients are overweight or obese (Table 2).

Among diabetic patients on medication 6 (8.6%) patients were found to be alcoholics and 64 (91.4%) patients were not. Among diabetic patients (control group), 10 (28.6%) patients were alcoholics and 25 (71.4%) were non drinker of alcohol.

The percentages of patients taking different anti diabetic drugs were different within the study group. 34 (48.6%) patients received insulin, 24 (34.3%) received metformin & glibenclamide, 5 (7.1%) received insulin & metformin, 2 (2.9%) received metformin and 5 (7.1%) received glibenclamide. The concentration of the drugs were metformin (500-2000 mg/dl), glibenclamide (2.5- 20mg/dl), insulin with various concentration. Gilibenclamide and insulin were taken BID 20-30 min before food while metformin 30 min after food (Table 2).

Table 2:- Demographic and clinical characteristics of type 2 diabetic patients who are receiving different anti-diabetic drugs and diabetic patients who don't receive any medication [Controls].

Variable	**patients with anti-diabetic drugs (n=70)**	**Patients without anti-diabetic drug (n=35)**	***p-value***
Age (years)			
Mean ± SD	55.10 ± 10.227	52.17 ± 11.833	
Range	34 – 76	28 – 78	***0.192***
Sex distribution			
Males	30 (42.9%)	19 (54.3%)	
Females	40 (57.1%)	16 (45.7%)	***0.268***
Duration of diabetes (years) Mean ± SD	12.33 ± 8.018	0(0)	
Range	1 – 40		
BMI (Kg/m^2)			
Underweight	1 (1.4%)	1 (2.9%)	
Normal	26 (37.1%)	15 (42.9%)	
Overweight	30 (42.9%)	15 (42.9%)	***0.199***
Obese	13 (18.6%)	4 (11.4%)	
Alcohol intake status			
Drinker	6 (8.6%)	10 (28.6%)	
Non-drinker	64 (91.4%)	25 (71.4%)	***0.007***
Treatment			
Insulin injection	34 (48.6%)	0(0)	
Insulin + metformin	5 (7.1%)	0(0)	
Glibenclamide	5 (7.1%)	0(0)	
Gibenclamide+ metformin	24 (34.3%)	0(0)	
Metformin	2 (2.9%)	0(0)	

As shown in Table 3, mean values of liver enzyme (ALT, ALP) and FBS, lipid profile (TC, TG, HDL, LDL) were lower in type 2 diabetic patients receiving metformin group than insulin receiving group. The mean value of BMI, TP, AST, AL, and TB among insulin group is lower than metformin group; but not statistically significant at the *p* value < 0.05 by using independent-t test analysis.

Table 3:- Biochemical characteristics of patients who were receiving metformin and insulin.

Parameters	**Type of drugs Mean ± SD**		
	Metformin (n=2)	**Insulin (n=34)**	***P- value***
BMI(Kg/m^2)	29.6 ± 0.28	27.9±4.9	***0.630***
FBS(mg/dl)	133 ± 7.1	219.3± 0.9	***0.146***
AST(U/l)	24 ± 2.83	20.38 ± 6.0	***0.408***
ALT(U/l)	15 ± 2.83	16.88±8.88	***0.769***
ALP(U/l)	194.5 ± 57.3	252.6±90.9	***0.382***
TP (g/dl)	8.15 ± 0.35	7.46 ± 0.65	***0.146***
AL (g/dl)	5.1 ± 0.42	4.5 ± 0.56	***0.156***
TB (mg/dl)	0.63 ± 0.30	0.57 ± 0.15	***0.650***
TC(mg/dl)	148 ± 60.81	182.1±36.5	***0.219***
TG (mg/dl)	112 ± 32.53	167.3±81.5	***0.350***
HDL(mg/d)	41.5 ± 6.36	47.62±9.62	***0.380***
LDL(mg/dl)	84 ± 48.08	104.4±34.6	***0.429***

*The mean is significant at the $p < 0.05$ (by independent t-test analysis)

Remarks: FBS =fasting blood glucose, AST = aspartate aminotransferase, ALT = alanine aminotransferase, ALP = alkaline phosphatase, TB = total bilirubin, TC= total cholesterol, HDL = high density lipoprotein, LDL = low density lipoprotein, AL = albumin, TP = total protein

The difference mean values of liver enzymes (AST, ALT, and ALP), TP, AL, lipid profile (TC, HDL and TG), BMI and FBS were not significant between type 2 diabetic patients receiving insulin and glibenclamide group. However there is an increase in the levels of AST, ALT, TP, AL and TG level in glibenclamide treated patients than insulin treated ones. On the contrary, FBS, TC, TB and HDL levels were increased in insulin treated patients but mean value of LDL in glibenclamide receiving group was lower than insulin receiving group significantly using independent- t test analysis as shown in Table 4.

Table 4:- Biochemical characteristics of patients who were receiving insulin and glibenclamide.

Parameters	**Type of drugs Mean ± SD**		
	Insulin (n=34)	**Glibenclamide (n=5)**	***P- value***
BMI(Kg/m^2)	27.9±4.9	27.3 ± 3.1	***0.787***
FBS(mg/dl)	219.3± 0.9	171 ± 41.29	***0.202***
AST(U/l)	20.38 ± 6.0	30.4 ±11.96	***0.053***
ALT(U/l)	16.88±8.88	26.4 ± 25.12	***0.099***
ALP(U/l)	252.6±90.9	250.4±103.2	***0.960***
TP (g/dl)	7.46 ± 0.65	7.8 ± 0.70	***0.252***
AL (g/dl)	4.5 ± 0.56	4.8 ± 0.35	***0.272***
TB (mg/dl)	0.57 ± 0.15	0.56 ±0.15	***0.849***
TC(mg/dl)	182.1±36.5	156.4±49.35	***0.167***
TG (mg/dl)	167.3±81.5	210.8±148.2	***0.325***
HDL(mg/d)	47.62±9.62	38.8 ± 16.28	***0.089***
LDL(mg/dl)	104.4±34.6	67 ± 16.38	***0.024***

The difference in mean values of liver enzymes (AST, ALT and ALP), TP, AL, lipid profile (TC, HDL, LDL and TG) BMI and FBS were not statistically significant between type 2 diabetic patients receiving metformin and glibenclamide group at *P* value <0.05 by using independent- t test analysis. However there was increment in liver enzymes, FBS, TC, and TG levels but decrease in TP, AL, HDL and LDL in glibenclamide receiving group than metformin receiving group (Table 5).

Table 5:- Biochemical characteristics of patients who were receiving metformin and glibenclamide.

Parameters	**Type of drugs Mean ± SD**		
	Metformin (n=2)	**Glibenclamide (n=5)**	***P- value***
BMI(Kg/m^2)	29.6 ± 0.28	27.3 ± 3.1	***0.367***
FBS(mg/dl)	133 ± 7.1	171 ± 41.29	***0.275***
AST(U/l)	24 ± 2.83	30.4 ±11.96	***0.763***
ALT(U/l)	15 ± 2.83	26.4 ± 25.12	***0.570***
ALP(U/l)	194.5 ± 57.3	250.4±103.2	***0.517***
TP (g/dl)	8.15 ± 0.35	7.8 ± 0.70	***0.569***
AL (g/dl)	5.1 ± 0.42	4.8 ± 0.35	***0.376***
TB (mg/dl)	0.63 ± 0.30	0.56 ±0.15	***0.696***
TC(mg/dl)	148 ± 60.81	156.4±49.35	***0.854***
TG (mg/dl)	112 ± 32.53	210.8±148.2	***0.416***
HDL(mg/d)	41.5 ± 6.36	38.8 ± 16.28	***0.836***
LDL(mg/dl)	84 ± 48.08	67 ± 16.38	***0.470***

Both insulin and insulin plus metformin treated patients are overweight but the latter had a lower blood glucose level than the former. Serum liver enzyme levels were lower in the insulin treated groups than those receiving combination therapies but difference was not statistically significant except AST. The TC level was significantly lowered in insulin treated patients than the group receiving combination therapy. The TG, TP, TB, and LDL cholesterol levels were also the same reduced but not to a statistically significant level (Table 6)

T**able 6:- Biochemical characteristics of patients who were receiving insulin and insulin plus metformin**.

Parameters	**Type of drugs** **Mean ± SD**		
	Insulin (n=34)	**Insulin & metformin (n=5)**	***P value***
BMI(Kg/m^2)	27.9±4.9	26.3 ± 4.2	***0.487***
FBS**(**mg/dl)	219.3± 0.9	160.4 ± 43.9	***0.123***
AST(U/l)	20.38 ± 6.0	27.4 ± 10.71	***0.035***
ALT(U/l)	16.88±8.88	22 ± 14.98	***0.279***
ALP(U/l)	252.6±90.9	269.4 ± 27.3	***0.686***
TP (g/dl)	7.46 ± 0.65	7.58 ± 0.52	***0.686***
AL (g/dl)	4.5 ± 0.56	4.6 ±0.71	***0.745***
TB (mg/dl)	0.57 ± 0.15	0.59 ± 0.12	***0.761***
TC(mg/dl)	182.1±36.5	218.2±37.24	***0.047***
TG (mg/dl)	167.3±81.5	197.4±104.5	***0.460***
HDL(mg/d)	47.62±9.62	44.2 ± 16.21	***0.502***
LDL(mg/dl)	104.4±34.6	129 ± 41	***0.156***

The mean values of liver enzymes (AST, ALT) and AL were significantly higher in type 2 diabetic patients receiving combination drug of glibenclamide plus metformin receiving group than insulin receiving group. In contrast mean value of BMI, among insulin receiving group is higher than glibenclamide plus metformin receiving group and statistically significant. Other than ALP and FBS, Mean value of TP, TB, TG, LDL, TC, HDL and LDL were lower in insulin receiving group but not significant. By using independent- t test analysis as shown in Table 7.

Table 7:- Biochemical characteristics of patients who were receiving insulin and metformin plus glibenclamide.

Parameters	**Type of drugs** **Mean ± SD**		
	Insulin (n=34)	**Metformin& glibenclamide (n=24)**	***P value***
BMI(Kg/m^2)	27.9±4.9	24.7 ± 3.5	***0.007***
FBS(mg/dl)	219.3± 0.9	197.3 ± 61.6	***0.269***
AST(U/l)	20.38 ± 6.0	26.92 ± 13.61	***0.016***
ALT(U/l)	16.88±8.88	28.67 ± 19.89	***0.003***
ALP(U/l)	252.6±90.9	244.5 ± 90.1	***0.737***
TP (g/dl)	7.46 ± 0.65	7.78 ± 0.76	***0.083***
AL (g/dl)	4.5 ± 0.56	4.9 ± 0.62	***0.016***
TB (mg/dl)	0.57 ± 0.15	0.61 ± 0.16	***0.364***
TC(mg/dl)	182.1±36.5	200 ± 34.45	***0.065***
TG (mg/dl)	167.3±81.5	193 ± 86.71	***0.254***
HDL(mg/d)	47.62±9.62	48.63 ± 11.61	***0.719***
LDL(mg/dl)	104.4±34.6	113.1 ± 43.17	***0.399***

Even though, mean values difference of liver and lipid profiles were not statistically significant between type 2 diabetic patients receiving metformin group and combination drug of metformin plus glibenclamide group, liver enzymes, lipid profiles and FBS were lowered in the group taking metformin than those taking metformin plus glibenclamide but TP, TB and AL were higher in metformin receiving group. With regards to BMI, the group taking combination drug had a mean value in the normal range but the metformin taking group was obese (see Table 8).

Table 8:- Biochemical characteristics of patients who were receiving metformin and metformin plus glibenclamide.

Parameters	**Type of drugs** **Mean ± SD**		
	Metformin (n=2)	**Metformin & glibenclamid (n=24)**	***P value***
BMI(Kg/m^2)	29.6 ± 0.28	24.7 ± 3.5	***0.063***
FBS(mg/dl)	133 ± 7.1	197.3 ± 61.6	***0.160***
AST(U/l)	24 ± 2.83	26.92 ± 13.61	***0.769***
ALT(U/l)	15 ± 2.83	28.67 ± 19.89	***0.350***
ALP(U/l)	194.5 ± 57.3	244.5 ± 90.1	***0.453***
TP (g/dl)	8.15 ± 0.35	7.78 ± 0.76	***0.510***
AL (g/dl)	5.1 ± 0.42	4.9 ± 0.62	***0.660***
TB (mg/dl)	0.63 ± 0.30	0.61 ± 0.16	***0.909***
TC(mg/dl)	148 ± 60.81	200 ± 34.45	***0.061***
TG (mg/dl)	112 ± 32.53	193 ± 86.71	***0.208***
HDL(mg/d)	41.5 ± 6.36	48.63 ± 11.61	***0.406***
LDL(mg/dl)	84 ± 48.08	113.1 ± 43.17	***0.371***

As presented in Table 9, mean values difference of liver enzymes (AST, ALT and ALP), TP, AL, lipid profiles (TC, HDL, LDL and TG), BMI and FBS were not statistically significant between type 2 diabetic patients receiving metformin group and combination drug of metformin plus insulin. But serum liver enzyme and lipid profile were higher in combination receiver than mono therapy receiver group.

Table 9:- **Biochemical characteristics of patients who were receiving metformin and insulin plus metformin.**

Parameters	**Type of drugs** **Mean ± SD**		
	Metformin (n=2)	**Insulin & metformin (n=5)**	***P value***
BMI(Kg/m^2)	29.6 ± 0.28	26.3 ± 4.2	***0.336***
FBS(mg/dl)	133 ± 7.1	160.4 ± 43.9	***0.440***
AST(U/l)	24 ± 2.83	27.4 ± 10.71	***0.692***
ALT(U/l)	15 ± 2.83	22 ± 14.98	***0.560***
ALP(U/l)	194.5 ± 57.3	269.4 ± 27.3	***0.053***
TP (g/dl)	8.15 ± 0.35	7.58 ± 0.52	***0.225***
AL (g/dl)	5.1 ± 0.42	4.6 ±0.71	***0.411***
TB (mg/dl)	0.63 ± 0.30	0.59 ± 0.12	***0.837***
TC(mg/dl)	148 ± 60.81	218.2±37.24	***0.108***
TG (mg/dl)	112 ± 32.53	197.4±104.5	***0.330***
HDL(mg/d)	41.5 ± 6.36	44.2 ± 16.21	***0.836***
LDL(mg/dl)	84 ± 48.08	129 ± 41	***0.262***

As presented in Table 10, Mean values of ALT, TP, AL, TB, and HDL, BMI were lower in combination drug of metformin plus glibenclamide receiving group than insulin plus metformin receiving group, while AST, ALP, TC, LDL, TG and FBS were lowered in insulin plus metformin receiving group but not significant by using independent- t test analysis.

Table 10:- Biochemical characteristics of patients who were receiving insulin plus metformin and metformin plus glibenclamide.

Parameters	**Type of drugs** **Mean ± SD**		
	Insulin & metformin (n=5)	**Metformin & glibenclamid (n=24)**	***P value***
BMI(Kg/m^2)	26.3 ± 4.2	24.7 ± 3.5	***0.369***
FBS(mg/dl)	160.4 ± 43.9	197.3 ± 61.6	***0.216***
AST(U/l)	27.4 ± 10.71	26.92 ± 13.61	***0.940***
ALT(U/l)	22 ± 14.98	28.67 ± 19.89	***0.487***
ALP(U/l)	269.4 ± 27.3	244.5 ± 90.1	***0.549***
TP (g/dl)	7.58 ± 0.52	7.78 ± 0.76	***0.575***
AL (g/dl)	4.6 ±0.71	4.9 ± 0.62	***0.346***
TB (mg/dl)	0.59 ± 0.12	0.61 ± 0.16	***0.830***
TC(mg/dl)	218.2±37.24	200 ± 34.45	***0.298***
TG (mg/dl)	197.4±104.5	193 ± 86.71	***0.920***
HDL(mg/d)	44.2 ± 16.21	48.63 ± 11.61	***0.470***
LDL(mg/dl)	129 ± 41	113.1 ± 43.17	***0.458***

The difference mean values of AST, ALP, TP, TG and BMI were lower in combination of metformin plus glibenclamide receiving group than glibenclamide receiving group but not statistically significant. But mean value of LDL and TC in combination of drug metformin plus glibenclamide receiving group were significantly higher than glibenclamide receiving group by using independent- t test analysis as shown in Table 11.

Table 11:- Biochemical characteristics of patients who were receiving metformin plus glibenclamide and glibenclamide.

Parameters	**Type of drugs** **Mean ± SD**		
	Metformin & glibenclamide (n=24)	**Glibenclamide (n=5)**	***P value***
BMI(Kg/m^2)	24.7 ± 3.5	27.3 ± 3.1	***0.130***
FBS(mg/dl)	197.3 ± 61.6	171 ± 41.29	***0.370***
AST(U/l)	26.92 ± 13.61	30.4 ±11.96	***0.667***
ALT(U/l)	28.67 ± 19.89	26.4 ± 25.12	***0.826***
ALP(U/l)	244.5 ± 90.1	250.4±103.2	***0.897***
TP (g/dl)	7.78 ± 0.76	7.8 ± 0.70	***0.922***
AL (g/dl)	4.9 ± 0.62	4.8 ± 0.35	***0.733***
TB (mg/dl)	0.61 ± 0.16	0.56 ±0.15	***0.516***
TC(mg/dl)	200 ± 34.45	156.4±49.35	***0.024***
TG (mg/dl)	193 ± 86.71	210.8±148.2	***0.715***
HDL(mg/d)	48.63 ± 11.61	38.8 ± 16.28	***0.119***
LDL(mg/dl)	113.1 ± 43.17	67 ± 16.38	***0.028***

As presented in Table 12, mean values of ALP, AL, TP and FBS were significantly higher in type 2 diabetic patients receiving anti-diabetic drug than in diabetic patients who don't receive any drug control group. In contrast, mean value of liver enzymes (AST, ALT) among study group were significantly lower than control grouped. Mean values of lipid profiles (TC, LDL) were significantly lower in type 2 diabetic patients receiving anti-diabetic drug than in the control group. Mean value of TB, TG and HDL were statistically not significant by using independent- t test analysis.

Table 12:- Mean values of the biochemical parameters in type 2 diabetic patients who are receiving anti-diabetic drugs and control group.

Parameters	**Patients with anti-diabetic drug (n= 70)**	**Patients without anti-diabetic drug (n=35)**	**P value**	**95% CI**
FBS	201.643 ± 71.390	132.743 ± 31.432	**< 0.001**	(43.79 – 94.01)
AST (U/l)	23.943 ± 11.853	35 ± 17.009	**< 0.001**	(-16.711) – (-5.404)
ALT(U/l)	22.186 ± 16.557	38.23 ± 18.052	**< 0.001**	(-23.049) – (-9.036)
ALP(U/l)	249.19 ± 86.59	183.543 ± 65.362	**< 0.001**	(32.71 – 98.57)
TP (g/dl)	7.623 ± 0.688	7.169 ± 1.056	**0.009**	(0.115 – 0.79)
AL (g/dl)	4.687 ± 0.598	4.28 ± 0.782	**0.004**	(0.135 – 0.68)
TB (mg/dl)	0.587 ± 0.155	0.529 ± 0.249	**0.149**	(-0.021) – (0.136)
TC (mg/dl)	188.014 ± 39.515	209.800 ± 52.246	**0.032**	(-36.2) – (-1.7)
TG (mg/dl)	179.77 ± 89.269	167.857 ± 79.286	**0.505**	(-23.44) – (47.27)
HDL(mg/dl)	46.814 ± 11.306	44.26 ± 11.016	**0.255**	(-1.95) – (7.26)
LDL(mg/dl)	105.914 ± 39.026	129.06 ± 42.846	**0.007**	(-39.7) – (-6.59)

Variables (factors) which may assume to affect the dependent variables (LFTs) were like age, BMI and Diabetic Duration (DD). However, these factors had no significant strong correlation with most of the LFTs and lipid profile (liver biomarkers). But, there was significant moderate negative correlation between: ALT and DD (r= - 0.389, *p* <0.001 (Figure3*)*, AST and DD (r= - 0.338, *P* <0.001 (figure 2).

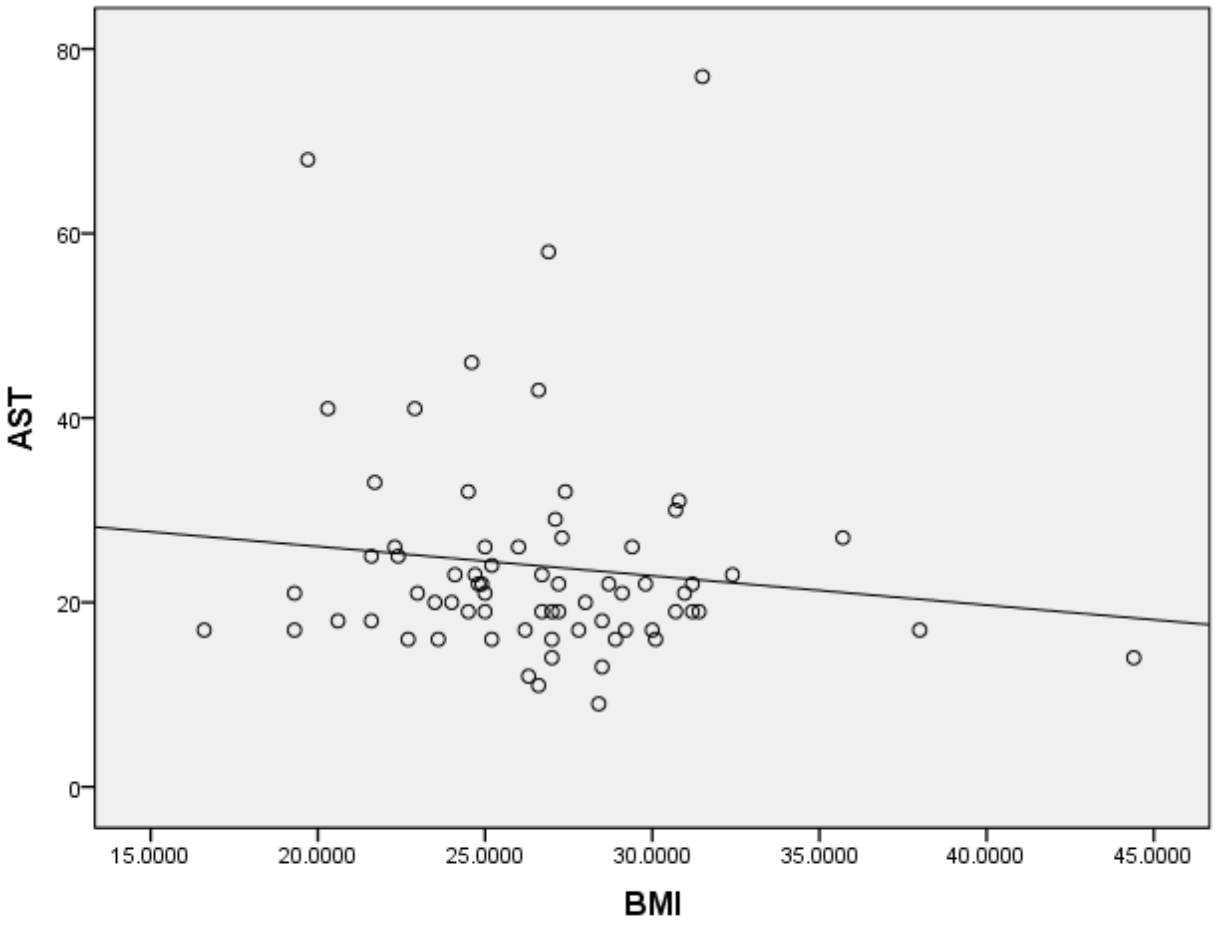


figure 1: The correlation of BMI with AST of pateints

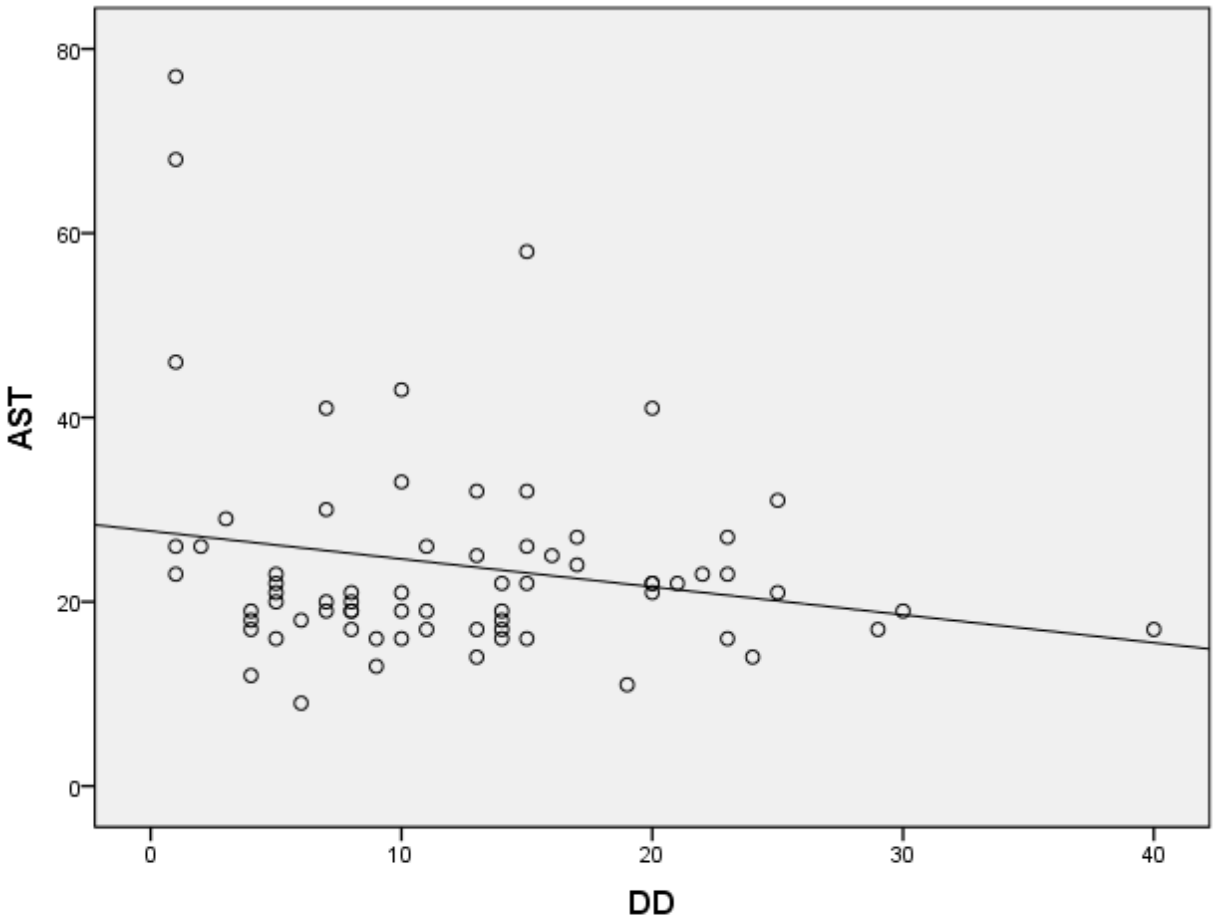


figure 2: The correlation of DD with AST of pateints

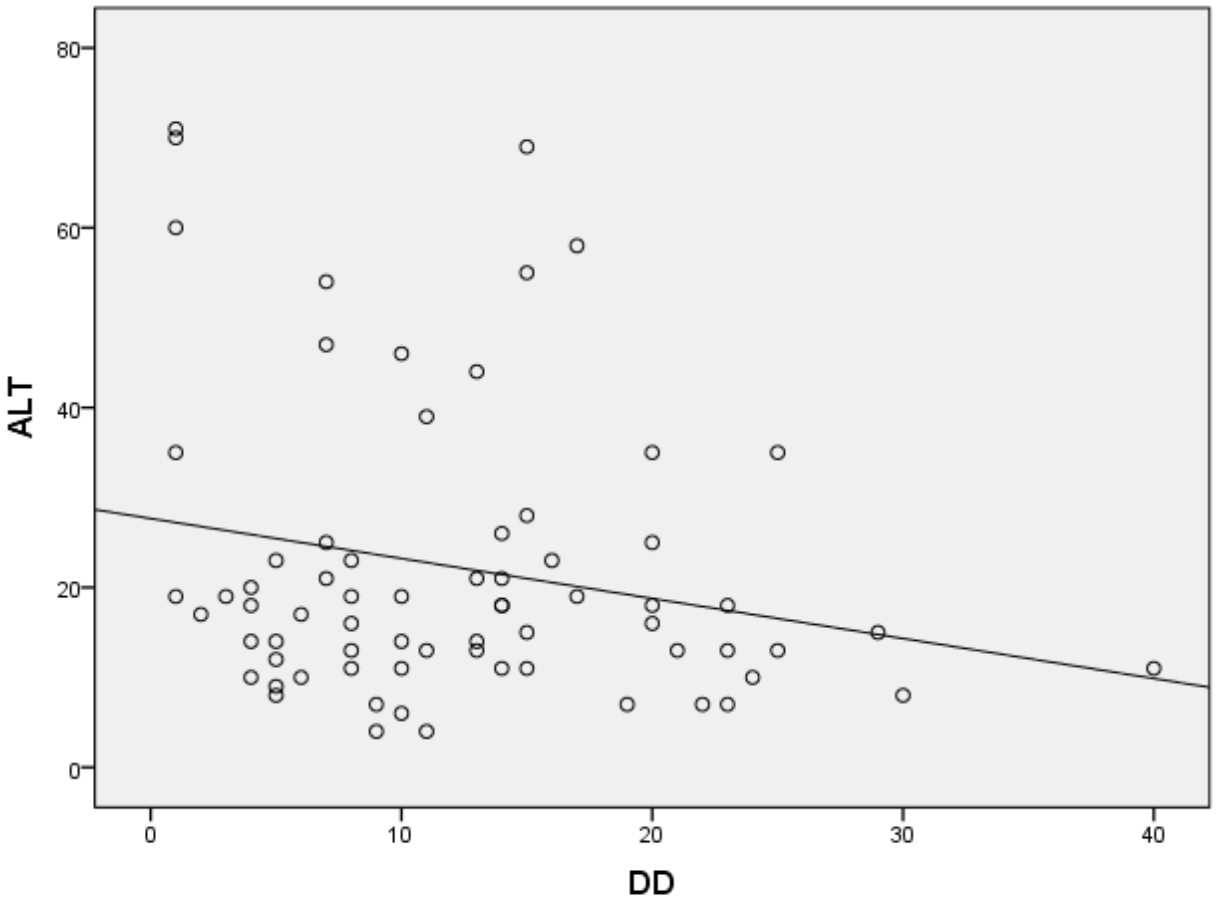


figure 3: The correlation of DD with ALT of pateints

6. Discussion

Diabetes is the most common metabolic disorder worldwide and has a high prevalence in developing countries; it is common in Ethiopia [1].Type 2 diabetes patients have been reported to be associated with higher incidence of abnormal liver function tests (LFT) compared to the individuals without diabetes, elevated ALT being the most common abnormality [7].

Many therapeutic drugs for diabetes target both fasting and postprandial hyperglycemia and other metabolic parameters involved in the diabetes-associated complications. These drugs are directed towards increasing insulin secretion, decreasing insulin resistance, and increasing insulin penetration into the cells. First generation Sulphonylureas were known for hepatotoxicity. The newer drugs in the thiazolidinediones class have a much larger margin of safety for liver toxicity. Very rare reports of liver toxicity, usually milder and reversible, have been seen with these drugs [8].

In present study as shown in Table 3, mean values of liver enzymes (ALT and ALP) were higher in type 2 diabetic patients receiving insulin group than metformin group but not significant. On the contrary, mean value of AST and TB were higher in metformin receiving group than insulin receiving group. The present results were in disagreement with the result reported by Swislocki and North found that metformin increase ALP, ALT and AST without any change in bilirubin. It may be due to differences in the background of patients in both situations (environment, genetic) and sample size [82]. Similarly other study reported by Al-Mola and Ahmed [79] showed ALP and ALT were higher ($p<0.05$) in metformin treated diabetics. At the same time AST and bilirubin did not show any changes. In contrast, Desilets *et al.,* [82] showed elevation of bilirubin in diabetic patient treated by metformin. However, this elevation in bilirubin returned to normal level after metformin was withdrawn.

The difference in mean values of liver enzymes was not significant between type 2 diabetic patients receiving insulin and glibenclamide groups. However there is an increase in the levels of liver enzymes, AL and TP in Glibenclamide treated patients than in insulin treated one as shown in (Table 4). On contrary, FBS were higher in insulin receiving group than glibenclamide receiving group. This could be due to an inject able of insulin which lead to non-compliance of patients.

In the present work as shown in Table- 5 serum liver enzyme levels were higher and TP, AL, TB were lower in the glibenclamide treated groups than those receiving metformin but difference was not statistically significant.

In present study as shown in (Table- 6) both insulin and insulin plus metformin treated patients are overweight. Serum liver enzyme levels, AL, TP and TB were higher in the insulin plus metformin combination drugs treated groups than those receiving insulin alone but difference was not statistically significant. Similarly Serum liver enzyme levels were higher in the insulin plus metformin treated groups than those receiving metformin but AL, TP, TB were higher in metformin receiving group but not statistically significant (Table-9). This could be due to number of population participated in this study. Likewise mean value of AST and ALP were higher in insulin plus metformin received group than metformin plus glibenclamide receiver group while ALT was lower in metformin plus insulin group (not significant Table-10). The mean values of AST, AL and ALT were significantly lower in type 2 diabetic patients that received insulin than the group that received combination drug of glebenclamide plus metformin group (Table-7). Similarly mean value of TP, TB, and lipid profile were lower in insulin receiving group but not statistically significant.

Serum liver enzymes (AST, and ALP) as well as TP levels were higher in the glibenclamide treated groups than those receiving metformin plus glibenclamide combination drugs but not statistical (Table-11). This may be due to the number of patients including in this study group. On the contrary, TB and AL were lower in glibenclamide receiver group. Serum liver enzyme levels were higher in the glibenclamide plus metformin treated groups than those receiving metformin but difference was not statistically significant (Table-8). Mean value of AL, TP and TB were lower in combination of glibenclamide plus metformin receiver than metformin alone receiver group.

Hepatic fat accumulation is a well-recognized complication of diabetes with a reported frequency of 40–70%. Fat is stored in the form of triglyceride and may be a manifestation of increased fat transport to the liver, enhanced hepatic fat synthesis, and decreased oxidation or removal of fat from the liver [1].

While most type 2 diabetic patients can be managed with oral therapy, the relentless decline in beta-cell function often leads to the eventual need for insulin therapy, either alone or in addition to oral agents [87].

In present study as shown in Table-6, TC level was significantly lowered in insulin treated patients than the group receiving combination therapy insulin plus metformin. The TG and LDL levels were also the reduced, but not to `statistically significant level. On the contrary HDL was increased in insulin alone receiver group. Similar results were reported by Mullugeta *et al*., [17] in which the patients on insulin therapy appeared to have slightly lower cholesterol levels but addition of metformin to the management protocol resulted in a significant improvement in the serum cholesterol. Addition of metformin not only decreased the total cholesterol levels but also had a positive effect on the distribution of cholesterol between HDL (increase) and LDL

lipoproteins (decrease).The results were in agreement with the Combined therapy with metformin plus bedtime insulin injections that showed beneficial effects on decreasing levels of LDL [87]. But mean values of lipid profiles (TC, LDL and TG) were higher among patients receiving combination therapy of insulin plus metformin than those receiving metformin plus glibenclamide receiving group but not statistically significant. In contrast, the mean value of HDL was lower in insulin plus metformin group. Even though it was not statistically significant as shown in Table-3, mean values of lipid profile (TC, TG, HDL and LDL) were higher in type 2 diabetic patients receiving insulin group than metformin group. This could be due to non-compliance of patients. The present work is not in agreement with the reported values of insulin therapy by Keidan *et al.,*[87] that showed LDL and TC levels were static but HDL levels were elevated. It was found that insulin therapy do not beneficially affect lipid levels leading to a reduction in triglyceride levels. Metformin was found to be effective in reducing insulin resistance and several studies were undertaken to assess its effects on total cholesterol (TC), triglycerides (TG), and HDL-cholesterol (HDL-C) levels. The literature shows various contradictory results about the influence of metformin on lipid profile. Some studies reported reduction only in TC levels, while others reported reduction of TC and TG levels with an increase of HDL-C [80]. Santana *et al.*, [81] have shown that treatment with metformin increased HDL level while serum total cholesterol levels were reduced and this is not in conformity with the present work. But, some similarities exist with decreased values of LDL. Mean value of TG, LDL, TC and HDL were lower in insulin alone receiver group than combination of metformin plus glibenclamide receiver group but not significant (Table- 7). The present work is not in agreement with the study which reported the combination of bedtime insulin plus daytime sulphonylureas. They showed similar lipid effects to those seen with insulin therapy alone.

The decrease in triglyceride, increase in HDL levels and LDL levels being constant with insulin therapy denotes dyslipidemia and shows similarities in insulin treated group with that of sulphonylurea treated groups [87]. Another result also reported by Mullugeta *et al*., [17] in which the patients on insulin therapy showed LDL higher than glibenclamide plus metformin therapy. The mean values of TC, TG and HDL levels were decreased in insulin treated patients than glibenclamide treated group but not statistically significant. On the contrary, value of LDL in insulin receiver group is higher than glibenclamide group which was statistically significant as shown in Table 4. Mullugeta *et al*., [17] reported similar results in which higher total cholesterol concentrations in the patients taking glibenclamide only. But, the present work is not in agreement with their report lowered LDL and HDL in glibenclamide received group than insulin group. The present results also in agreement with the study reported by Rivellese *et al.,*[16] that showed insulin therapy compared with glibenclamide is associated with decreases in plasma triglyceride, but not in agreement with their report on the increased level of high density lipoprotein in insulin therapy and no change in low density lipoprotein. It could be suggested that non-compliance of the patients with reference to insulin therapy.

Even though it was not statistically significant, it was shown in the present work that mean value of BMI, in group taking metformin was higher than those groups taking other drugs but the FBS was better controlled in the group taking metformin. The present study do not support the concept that the metformin treatment avoids weight gain in diabetic patients that emphasized the advantageous and uniqueness of metformin in avoiding the weight gain associated with other pharmacological treatments of type 2 diabetics. This may be due to the number of population in this study group. Avile´s-Santa *et al*., [84] reported a 0.5-kg weight gain in subjects taking insulin plus metformin. The insulin plus placebo subjects in their study gained an average of 3.2

kg. Although metformin has anorexic properties, the precise reason metformin treated diabetic patients do not gain weight is still unclear.

The difference in mean values of TC and TG were lower in metformin therapy than glibenclamide where as HDL and LDL was higher in metformin receiver group but not statistically significant (Table 5). Similar result was reported by Al-neaimy K.S.A [78] which showed an improvement in all lipid profile parameters by metformin therapy, but the improvement did not reach statistical significance, it could be due to non-compliance and the number of patients included in this study, while in the group that received the glibenclamide therapy, there were improvement in the lipid profile, and specially a significant reduction in TC, and LDL-C which disagreed with our result. The present study was in agreement with the reported result by Mughal *et al.,* [85] that showed total cholesterol, triglycerides, low-density lipoprotein and very low density lipoprotein did not change significantly during glibenclamide therapy.

As presented in Table 8, mean values of lipid profile were lower in metformin alone therapy than metformin plus glibenclamide combination therapy but not statistically significant. Mean value of LDL and TC in combination drug of metformin plus glibenclamide receiver group was significantly higher than glibenclamide alone receiver group. The mean difference of HDL and TG were not significant; however, mean value of TG was higher in glibenclamide alone receiver group (Table-11). This may be due to duration of follow up. Similarly, results reported by Garber *et al.,* [86] showed that patients administered with glibenclamide plus metformin tablets had increase in HDL and LDL levels than patients receiving mono therapy of Glibenclamide or metformin. This reported value was not in agreement with the present data in which mean value of TG was higher in glibenclamide mono therapy.

Our results have emphasized variables (factors) which may assume to affect the dependent variables (LFTs) like age, BMI and Diabetic Duration (DD). However, these factors had no significant strong correlation with most of the LFTs and lipid profiles (liver biomarkers). But, there was significant moderate negative correlation between: ALT and DD (r= - 0.389, *p* <0.001), AST and DD (r= -0.338, *P* <0.001) (Figure3). Similar findings were reported in the study by Belay *et al.,* [1] in which Variables which may assume to affect the dependent variables (LFTs and lipid profiles) were like waist to hip ratio (WHR), age, BMI and Diabetic Duration (DD). However, these factors had no strong correlation with most of the LFTs and lipid profile (liver biomarkers). Contrary to this situation, Ni *et al*., [7] found a significant positive correlation between ALT and BMI (r=0.555, *p*-value <0.001). Similarly, AST significantly increased with BMI showing significant positive correlation (r=0.431, *p*-value <0.001).

As presented in Table-12, mean values of ALP, TP, AL and FBS were significantly higher in type 2 diabetic patients receiving anti-diabetic drug than in diabetic patients who don't receive any drug (control group). Which was disagreeing with findings reported in a study by Patra T *et al.,* [8] in which the distribution level of alkaline phosphatases were greater in patients without drug group. This could be due to duration of diabetes in study group and most of the individuals in control group were pre-diabetics.

The mean value of liver enzymes (AST, ALT) among study group was significantly lower than control group at the p value < 0.05. Similar findings were reported in a study by Patra T *et al.,* [8] in which the mean values of AST and ALT among patients with drugs were lower than patients without drugs. Mean values of lipid profiles (TC, LDL) were significantly lower in type 2 diabetic patients receiving anti-diabetic drug than in the control group (P<0.05). Mean value concentrations of TG and HDL were statistically not significant at the p value<0.05. In the

present study, there was no significant difference among the distribution of TB levels between the two groups of population. But the average bilirubin level in 'patients without drugs' group is less than the patients with drugs' group (0.587 compared to 0.529; $p>0.05$). This result disagree with the finding reported in the study by Patra T *et al.,* [8] in which the mean values of TB level in patients without drugs' group is greater than the patients with drugs' group that were not significant.

7. Conclusion

This work confirms that anti-diabetic drugs were found to have an effect in lowering liver enzymes and lipid profiles in type 2 diabetic patients. The effects were found to be prominent in patients who were consuming drugs in comparison with groups of individuals who were not taking any medications. However, in patients tested in the present study, it has been observed that the effects of anti-diabetic drugs did not reduce levels of total protein, albumin and alkaline phosphatase. Many different biochemical parameters tested were more or less statistically similar in different groups of individuals who were on different anti-diabetic drugs.

8. Limitations and Recommendations of the study

The limitations in the present study include:-

- Small study population, lack of histopathological studies on the liver, and sequential pathological and anatomical studies on liver functions during the drug regimen could not be undertaken.
- Also, this short cross- sectional study could not follow up the patients, who were taking anti-diabetic drugs for long duration of biochemical and enzymatic progression due to limitation of money and time.
- The patients compliance to the drugs and the regimen of treatment could not be ascertained.

The following recommendations are forwarded:-

- Further study is needed with large sample size to investigate effects of anti-diabetic drugs on liver functions among T2DM in our country.
- More studies are needed which include techniques such as histopathological, pathological and anatomical parameters to understand more specific effects of the drugs
- More studies are needed with follow up on patients, in order to understand the progressive biochemical changes within the duration of drug regimens.
- Overestimation level of adherence to diabetic drugs is to be avoided and to monitor the process of strict drug compliance by the patients during treatment because they may breakup participating in the study.
- In future, interventions are urgently needed to improve adherence to anti-diabetic drugs in the study area.

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Appendix

Annex I:- Consent form in English

Purpose

Diabetic Mellitus is a common incurable chronic disease. During the past decade, DM has emerged as an important clinical and public health problem throughout the world. Likewise in Ethiopia, diabetes has become more common and it is a fast growing disease. Type 2 diabetes accounts for well over 90% of diabetes in Sub-Saharan Africa. The prevalence of liver diseases is higher in patients who have suffered from type 2 diabetes mellitus (T2DM. Hence, prevention or early diagnosis of progressive liver disease is needed in T2DM patients. The aim of this studying is to investigate profile of liver function tests among type 2 diabetic patients who are receiving different anti-diabetic drugs. Therefore, this study will reflect status of liver among those diabetes patients.

Participation

Without your participation, voluntarism and active part of the study the feasibility of the research are under question, so we asking you and all other to voluntary participant in this study. What we expect from you is to be willing to give blood to examine the status of your liver. The examination involves laboratory procedure with collection of 3ml blood from arm. Sample will be collected using sterile and disposable needles and test tubes.

Risks

Taking 3ml of blood does not have any harm to your health but minor needle pain may last for seconds. If there comes any discomfort, we shall offer you necessary medical treatment freely.

Benefits

Any status of examination will be communicated with your physician and facilitate appropriate measures need to be taken accordingly.

Confidentiality

Any information collected from you will be kept confidential. Your identity will not be disclosed in any situation or study result as we use different code number instead of your name.

Sharing the Result

After analysis of the data, we will present the result of the study to the responsible bodies. The report will not bear any information about you; because we use code to disseminate the results to concerned bodies and for the purpose of publication.

Right to Refuse

Since your participation in this study is entirely on voluntary of you, you have right to refuse to accept this request. Your refusal will not affect any part of your treatment in hospital concerning our study.

Contact Address

If you have any question or concern, you can contact Desaleng Dango at any time using the following address

Desaleng Dango, Addis Ababa University, Faculty of Medicine, Department of Medical Biochemistry.

Tel: 0924192852

Email: lmosisa2003@gmail.com

Addis Ababa, Ethiopia

Consent Form

I, the under signed, confirm that, as I give consent to participate in the study, it is with a clear understanding of the objectives and conditions of the study and with recognition of my right to withdraw from the study if I change my mind.

I……. do hereby give consent to Dr. /Mr. /Mrs. /Miss……..to include me in the proposed research. I have been given the necessary information about the research. I have also been assured that I can withdraw my consent at any time without penalty or loss of benefits. The proposal is explained to me in the appropriate language I understand.

Unique number of the Participant ……..

Signature of the Participant ……

Name of the Investigator……

Investigators Signature…….

Date: …….

Annex II: Amharic version

Addis Ababa University School of Medicine

Department of Biochemistry

<u>የስምምነት ማረጋገጫ ፎርም</u>

አላማ

ስኳር በሽታ የማይግል የሚታወቅ በሽታ ነው፡፡በዚሁ በ10 ዓመታት በአለማችን ዋነኛው የጤና የሕብረተሰብ ችግር ሆኗል፡፡እንዲሁም በኢትዮጵያ በየጊዜው እያደገ መጥቷል፡፡ የ2ኛ ደረጃ ስኳር በሽታ የመካከለኛው አፍሪካ 90 በመቶ ደረጃ ላይ ደርሷል፡፡ እነዚህ በ2ኛ ደረጃ ስኳር በሽታ የተተኩ ሰዎች በጉበት በሽታ በብዛት ይጠቃሉ፡፡ ስለዚህ ይህንን ጉበት በሽታ ሳይባባስ ማከም አስፈላጊ ነው፡፡ የዚህ ዋነኛው ጥናት ዓላማ የዚህ ጥናት አላማ የደረጃ ሁለት የስኳር ታማሚ ሆኖ የተለያየ መድሃኒት የሚወስዱትን የጉበታቸንን ሁኔታ ማጥናት ነው፡፡

ተሳትፎ

ይህ ጥናት ያለናንተ ተሳትፎና ፍላጎት ግቡን ይደርሳል ማለት አይቻልም፡፡ ስለዚህ ሁላችሁም በዚህ እንዲትሳተፉ እንጠይቃለን፡፡ እኛ ከእናንተ የምንፈልገው በፍላጎት የደም ናሙና 3 M.L. እንዲትሰጡን ነው፡፡

ጉዳት

3 M.L. **የጤና ችግርን ያመጣል ብለን አናምንም ነገር ግን በመርፌው ምክንያት ለትንሽ ሰከንድ ህመም ሊሰማችሁ ይችላል፡፡ ሌላ ችግርም ቢፈጠር የሚያስፈልገውን ህክምና በነፃ እንሰጣለን፡፡**

ፋይዳ

ጥናቱ የሚያሳየውን ውጤት አይተን ሐኪናችሁን ችግር ካለ በአግባቡ እንዲትታከሙ እንጠይቃለን፡፡

ሚ ስጢርን መከጠበቅ

ከእናንተ የተሰበሰበው ሁሉ በሚስጢር ይቀመጣል፡፡

በስማችውና በኮድ የያዘው ሚስጥር ካለ አግሞ ተቆልፎበት ይቀመጣል፡፡

እንቢ የማለት መብት

ይህ ጥናት በእናንተ ፍላጎት ላይ የተመሰረተ ስለሆነ እንቢ የማለት መብት አላችሁ፡፡ ይህ ደግሞ በእናንተ በምታገኙት ሕክምና ላይ ምንም ዓይነት ችግር አያደርስም፡፡

አድራሻ

ጥያቄ ወይም ሐሳብ ካላችሁ የፈለጋችሁት ስዓት ደሳለኝ ዳንኅ መገናኘት ትችላላችሁ፡፡

ደሳለኝ ዳንኅ ፡ አዲስ አበባ ዩኒቨርስቲ ፣ የሕክምና ፋካልቲ ፣ ባዮ ኬሚስትሪ ዲፓርትመንት

ስልክ +2519-24-19-28-52

ኢሜል lmosisa2003@gmail.com

አዲስ አበባ ፡ ኢትዮጵያ

የቃል ስምምነት

ከላይ የተፈውን የመረጃ ቅፅ እንብቤሁ የጥናቱን አላማና ጥቅም በግልፅ ተረድቻለሁ፡፡ በዚህም መሰረት ያለ ጥናት ቡድኑ አባላት ተፅኖ በሙሉ ፍቃደኝነት በዚሁ ጥናት ውስጥ በመሳተፍ የሚጠበቅብኝን አስተዋፅኦ ለማበርከት መወሰኔን በፊርማዬ አረጋግጣለሁ፡፡

የታካሚው መለያ ቁጥር _____ ፊርማ _____

የመረጃ ሰብሳቢ ሥም _____ ፊርማ _____

መረጃ የተሰበሰበበት ቀን_____የተጀመረበት ሠዓት ____ ያለቀበት ሰዓት _____

የተቆጣጣሪ ሥም _____ ፊርማ _____

ቀን_____

Assurance of the Principal Investigator

I, who undersigned, agree to accept responsibility for the scientific ethical and technical conduct of the research project and for the provision of required progress reports as per terms and conditions of the research publications office in effect at the time of grant is forwarded as the result of this publication.

Name of the student: _____

Date …… Signature…….

Assurance of Advisors

Name of the Advisors

1. Dr. Melaku Umeta

   Date ……Signature……

2. Dr. Tedla Kebed

   Date ……Signature……

3. Dr. Solomon Genet ,

Date ……Signature……

4. Dr. Menakath Menon

Date ……Signature……

Annex 3:- Sample collection sheet

Sample code		**Lipid profiles**				**LFTs**						**Sex**	
	FBS	**LDL**	**HDL**	**TG**	**TC**	**TB**	**AST**	**ALT**	**ALP**	**TP**	**AL**	**M**	**F**
S01													
S02													
S03													
s04													

Sample collection approval

Health professional Name …… signature……..

Principal Investigator Name **Desaleng Dango** signature ……

Advisor

Dr. Melku Umeta signature …….

Dr. Tedela Kebede signature …….

Dr. Solomon Genet signature …….

Dr. Menakath Menon signature …….

Annex 4:- Questionnaire

Dear respondents,

Given below are the items specifying necessary information expected from you. The questionnaire is a part of the study for the masters of degree at Addis Ababa university school of graduate studies. The objective of the research is to investigate profile of liver function test among type 2 diabetic patients who are receiving different anti diabetic drugs. This study is purely academic and all your responses will be used in strict confidentiality in accomplishing the requirements of the study. Your genuine answer for the questions in the questionnaire has an immense value to the completion of the study.

Part 1

Personal information: please make a circle" on the options that best describes you.

1. **Gender** : Female/Male
2. **Education**:

   A) high school B) diploma C)degree D) masters and above
3. **Age in year**: …….

4. **Alcoholic intake status**:

   A) drinker B) non-drinker

5. **If you are a drinker how often do you drink**?

   A) once a week B) twice a week C) monthly D) every day

   E) others ……

6. **Smoking status:**

   A) smoker B) non- smoker

Part 2

Health information: please make a circle" on the options that you choose

1. Do you have any health condition or disability (physical or mental problem

   A) yes B) no

2. Do you have any liver disease problem before?

   A) yes B) no

3. Have you taken HIV tests before?

   A) yes B) no

4. When your result was positive, have you taken any HIV drug ?

   A) yes B) no

5) How long you stay with type 2 diabetic disease? .....

6). Height……

Weight……..

Or BMI…….

Code; _____

Annex 5:- Questionnaire in Amharic

ለተሳታፊዎች

ከዚህ በታች የተዘረዘሩትን ጥያቄዎች ከእናንተ የሚፈለጉ ናቸው፡፡

ጥያቄው በአዲስ አበባ ዩኒቨርስቲ ለድህረ ምረቃ ጥናትን የሚያስፈልጉ ናቸው፡፡

የዚህ ጥናቱ አላማ የደረጃ ሁለት የስኳር ታማሚ ሆኖ የተለያየ መድሃኒት የሚወስዱትን የጉበታቸንን ሁኔታ ማጥናት ነው፡፡ የእናንተን መልስ መስጠት ይህ ጥናት አዓማ እንዲደርስ የእናንተ መልስ መስጠት አስፈላጊ ነው፡፡

ክፍል አንድ

ግላዊ ሚስጢር ፡- ከዚህ በታች እርስዎን የሚገልጽ ክብ አድርጉ

1. ፆታ ፡- ወንድ/ሴት
2. የትምህርት ደረጃ

   ሀ. ሁለተኛ ደረጃ ለ. ዲፕሎም ሐ ድግሪ ሠ. ማስተርና ከዚያ በላይ
3. ዕድሜ ----------
4. የመጠጥ ሁኔታ፡-

   ሀ. እጠጣለሁ ለ. አልጠጣም

5. የሚጠጡ ከሆነ የምን ጊዜያት ይጠጣሉ

   ሀ. በሳምንት 1 ጊዜ ለ. በሳምንት 2 ጊዜ ሐ. በወር 1 ጊዜ ሠ. ሁል ጊዜ ረ. ሌላ ካለ ----------

6. የማጨር ሁኔታ

   ሀ. አጨሳለሁ ለ. አላጨስም

ክፍል ሁለት

የጤና መረጃ ፡- ከዚህ በታች እርስዎንም የሚገልፅ ላይ ክብ ያድርጉ

1. የአእምሮ ችግር አለባችሁ ? ሀ. አዎ ለ. የለም
2. ከዚህ በፊት የጉበት በሽታ ችግር አለባችሁ ? ሀ. አዎ ለ. የለም
3. ከዚህ በፊት ኤች አይ ምርመራ አድርገው ያውቃሉ? ሀ. አዎ ለ. የለም
4. ውጤቱ ፖዘቲቭ ከሆነ መድሐኒት ይወስዳሉ ? ሀ. አዎ ለ. የለም
5. ከሁለተኛ ደረጃ ህመም ጋር ለስንት ዓመት ነው የቆዩት? ----------
6. ቁመት ----------

   ክብደት ----------

   ወይም BMI ----------

ኮድ ----------