ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF MEDICINE

DEPARTMENT OF BIOCHEMISTRY

COMPARISON OF LIPID PROFILE, LIVER ENZYMES, CREATINE KINASE AND LACTATE DEHYDROGNASE OF T2DM PATIENTS WHO WERE ON STATIN ATTENDING DIABETIC CLINIC OF TIKUR ANBESA SPECIALIZED HOSPITAL

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A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES OF ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENTS OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF SCIENCES IN MEDICAL BIOCHEMISTRY

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Comparison of lipid profile, liver enzymes, CK and LDH of T2DM patients who were on statin attending Diabetic Clinic of Tikur Anbessa Specialized Teaching Hospital

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This is to clarify that thesis prepared by Mezgebu Legesse entitled as, "comparison of lipid profile, liver enzymes, creatine kinase and lactate dehydrogenase of T2DM patients who were on statin attending diabetic clinic of Tikur Anbesa Specialized Hospital" is submitted in partial fulfillment of the Requirement for the Degree of Master of Sciences in Medical Biochemistry complies with the regulations of the university and meets the accepted standards with respect to the originality and quality.

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Chair of Department or Graduate Program Coordinator

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List of abbreviation

ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
CK	Creatine kinase
CVD	Cardiovascular disease
FPG	Fasting plasma glucose
GAD	Glutamic acid decarboxylase
GDM	Gestational diabetes mellitus
G6PDH	Glucose-6-phosphate dehydrogenase
HbA1C	Hemoglobin A1C
HDL	High density lipoprotein
HMG-CoA	3-hydroxy-3-methylglutaryl-CoA
IDF	International diabetes federation
LDH	Lactate dehydrogenase
LDL	Low density lipoprotein
NICE	National institute for health and care excellence
OGTT	Oral glucose tolerance test
PCSK9	Proprotein convertase subtilisin/kexin type 9
TASH	Tikur anbesa specialized hospital
T1DM	Type 1diabetes mellitus
T2DM	Type 2 diabetes mellitus

ABSTRACT

Background: Diabetes mellitus (DM) is an epidemic disease affecting millions worldwide; the majority being T2DM. DM has been shown to be an important risk factor in the development of a variety of cardiovascular conditions becoming common in Ethiopia. Consequently, risk-reducing statin therapy is recommended for nearly all patients with diabetes at 40 years of age or older, regardless of cholesterol level. But, there are controversy regarding its safety.

Objective: The aim of this study was to assess and compare the level of lipid profile, liver enzymes, creatine kinase-MB (CK-MB) and lactate dehydrogenase (LDH) in T2DM patients who were on statin therapy.

Methodology: Hospital based cross-sectional study was conducted on a total of 100 T2DM patients, divided into four groups (Group I, II, III and IV) each n = 25 T2DM patients who were on statin for 14 days–6months, 6–18months, >18months and who were not on statin therapy respectively. Convenient sampling technique was implemented till the required number has been achieved. Sociodemographic data was collected by using standardized questionnaire. Fasting blood was collected and lipid profile, liver enzymes, CK-MB, LDH and fasting blood sugar (FBS) were analyzed for all patients. Study was conducted from Mar - Sep, 2017.

Result: the mean value of total cholesterol and TAG were significantly lower among group III as compared to group I (*P- value*= 0.019 & 0.01). Similarly, LDL-c were significantly lower among group III as compared to group I (P=0.022) and group IV (P=0.027). In addition, mean values of lipid panel among the four study groups were higher than normal value. Serum ALT, AST and ALP, CK-MB and LDH were not show statistically significant differences among the study groups ($p > 0.05$). Mean value of ALT and AST were found within normal range while mean ALP was higher in all study groups. Fasting blood glucose was not different and but higher than normal values in all groups.

Conclusion: Statin taken for longer time has an effect in lowering total cholesterol, LDL-cholesterol and TAG in T2DM patients. CK-MB, LDH, liver enzymes (ALT, AST and ALP) and other parameters tested were more or less similar in all groups of study participants.

Key words: CK-MB, HMG-COA reductase, Lipid profile, Liver enzymes, Statin, T2DM, LDH

CHAPTER ONE

INTRODUCTION

1.1. Background

Diabetes is a serious, chronic disease that occurs either when the pancreas does not produce enough insulin (a hormone that regulates blood sugar, or glucose), or when the body cannot effectively use the insulin it produces. It is characterized by hyperglycemia with disturbances of carbohydrates, fat and protein metabolism (Borle *et al.*, 2016). Diabetes is one of the four prioritized non-communicable diseases (NCDs) targeted for action and the number of cases and the prevalence of diabetes have been steadily increasing over the past few decades. Globally, 108 million diabetic case in 1980 that increased to 422 million case in 2014. Over the past decade, diabetes prevalence has risen faster in low and middle-income countries than in high income countries. T2DM where the body cannot properly use the insulin it produces is the most prevalent form of diabetes (American Diabetes Association, 2016; Model, 2015). In 2015, there were 14.2 million diabetes cases in Africa and this number will reach to 34.2 million by 2040. In Ethiopia, occurrences and complications of DM have been increasing from time to time in an alarming rate (Mistire, 2013).

Heart attack, stroke, kidney failure, leg amputation, vision loss and nerve damage are the most common complications in diabetes and can increase the overall risk of dying prematurely. There are different effective approaches available to prevent T2DM and its complications. These includes regular physical exercise, eating healthy diet, avoid smoking, controlling blood pressure and lipid level. The starting point for living well with diabetes is an early diagnosis followed by glycemic control (Krikorian, 2016). One of the consequence of metabolic syndrome as a result of diabetes is dyslipidemia which is characterized by elevated fasting and postprandial triglycerides (TG), low level of high density lipoprotein cholesterol (HDL-C), elevated low density lipoprotein- cholesterol (LDL-C) and the predominance of small dense LDL particles. Dyslipidemia is defined as a disorder of lipoprotein metabolism, either overproduction or deficiency. Study indicate that diabetic dyslipidemia is caused by impaired action of lipoprotein lipase (LPL) that is localized to the endothelial cells. And these increase smaller size and modified LDL, such as glycated and oxidized LDL that play an important role to induce vascular and renal cellular dysfunction, cardiovascular risk and these fasten diabetic complication. Most of these effects are mediated by Rho-kinase inhibition (Kawanami *et al.*,

2016; Wu & Parhofer, 2014; Mooradian, 2009). Non-pharmacologic interventions (diet and exercise) are first line therapies and are adjuvant to the pharmacologic therapy when necessary in treatment of diabetic dyslipidemia. Lowering LDL-C level is the first priority in treating diabetic dyslipidemia mainly circulating levels of smaller and denser LDL particles. Because high plasma level of LDL-C contribute to plaque formation in the arteries that narrows blood vessels and restrict blood flows. From pharmacologic therapy, statins are the first drug of choice, followed by resins, ezetimibe, fenofibrate, niacin and others. If a single agent is inadequate to achieve lipid goals, combinations of the preceding drugs may be used (Catapano *et al***.,** 2016; Basak *et al*., 2013).

Cardiovascular diseases (CVDs) are disorders of heart and blood vessels that include coronary heart disease, cerebrovascular disease, rheumatic heart disease and other conditions. The risk assessment of CVD is recommended to guide clinicians in the selection of the most appropriate treatment, especially when a pharmacological therapy is needed. Targets for LDL-C level is very important in choice of a pharmacological lipid-lowering agent. LDL-C < 1.8 mmol/l (70 mg/dl) and TG < 2.3 mmol/l values are greatly desired for very high risk patients (Gencer & Mach, 2016).

Statin lowers atherogenic LDL-C through inhibition of 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductase, rate-limiting enzyme of cholesterol biosynthesis and upregulation of LDL receptors on the cell membrane. Thereby it reduces CVD by 25-30% (Rutishauser, 2006). Statin introduced as best cholesterol lowering drug since 1987 and it includes; atorvastatin, simvastatin, pravastatin and rosuvastatin (Collins *et al.*, 2016). In addition, statin has ant-inflammatory, immunomodulation and anti-microbial effects (Hennessy *et al.*, 2016; Joo, 2012). As a result, statins are considered very effective in reducing cardiovascular morbidity and mortality in high-risk patients like T2DM (Bitztur *et al.*, 2013).

Since most drugs are metabolized and processed in the liver, drug induced hepatotoxicity which is characterized by elevation of liver enzymes in the plasma is common. The three most important mechanisms by which drugs induce liver injury are direct cell stress, mitochondrial impairment and specific immune reactions (Singh *et al*., 2016; Russmann *et al.*, 2009). The effect of statins in reducing the risk of cardiovascular disease is well-established and it appears to be safe (Kalantari and Naghipour, 2014). But several clinical studies have demonstrated evidences of liver toxicity, mostly manifesting as minor and persistent elevations in liver aminotransferases (Russo *et al.*, 2014; Arca and Pigna, 2011).

Evaluation of liver enzymes or liver function tests (LFTs) are commonly used in clinical practices to screen for liver disease, monitor the progression of known disease and the effects of potentially hepatotoxic drugs. The most common LFTs include the serum aminotransferases, alkaline phosphatase, bilirubin, albumin, and prothrombin time. Aminotransferases; alanine amino transferase (ALT) and aspartate aminotransferase (AST), serve as a marker of hepatocyte injury whereas alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), and bilirubin are markers of biliary function and cholestasis (Choudhary and Vyas, 2015; Hall and Cash, 2012; Huang *et al*., 2006).

The other concern over statin safety is myopathy and rhabdomyolysis which is characterized by increased activity of CK and LDH (Stroes *et al.,* 2015; Maji *et al.*, 2013). Higher doses of statins have been linked to an increased risk of a life-threatening form of muscle breakdown called rhabdomyolysis. This can lead to permanent kidney damage, coma, and death. Symptoms include fatigue, muscle pain, muscle tenderness, muscle weakness, nocturnal cramping and tendon pain. The mechanism of statin induced myopathy could be impaired synthesis of cholesterol leading to changes in the cholesterol in myocyte membranes and behavior of the membrane, impaired synthesis of compounds in the cholesterol pathway (coenzyme Q10) and depletion of isoprenoids which prevents myofibril apoptosis (Ambapkar *et al*., 2016; Pedro-Botet *et al*., 2016; Tokinaga *et al*., 2006).

Measuring serum CK is an important part of the evaluation of patients with muscle weakness or myalgia, and assessing of patients with myopathies or muscle injuries. Drugs like statins and other supplements are an important and common cause for serum CK elevation (Moghadam-kia *et al*., 2016). Similarly, evaluation of serum LDH or LDH isoenzyme is relevant in the diagnosis, prognosis and monitoring of diseases such as myocardial infarction, hemolytic anemia, hepatocellular carcinoma, ovarian dysgerminoma and testicular germ cell tumor (Faloppi *et al.,* 2016; Del Prete *et al.,* 2016)

1.2. LITRATURE REVIEW

1.2.1. Burden of diabetes worldwide

Diabetes is a complex, chronic, debilitating and costly illness requiring continuous medical care with multifactorial risk-reduction strategies beyond glycemic control. Ongoing patient self-management, education and clinical supports are very important to prevent acute complications and reduce the risk of long-term complications (Cefalu, 2017). In 2015, 30.3 million (9.4%) of the U.S people of all ages had diabetes and the incidence is higher among older age (Centers for Disease Control and Prevention, 2017). WHO estimates that globally, over 422 million adults aged over 18 years were living with diabetes in 2014 and still it is increasing. Worldwide diabetes caused 1.5 million deaths in 2012 and incidence and death rate is become higher in low- and middle-income countries than in high-income countries. The largest numbers of people with diabetes estimated by the WHO were South-East Asia and Western Pacific Regions. Africa has the highest proportion of undiagnosed diabetes. The global prevalence of diabetes has grown from 4.7% in 1980 to 8.5% in 2014, during which time prevalence has increased or at best remained unchanged in every country. There were over 1.33 million cases of diabetes in Ethiopia in 2015 (World Health Organization, 2016).

Diabetes has imposed a great economic burden throughout the world directly for clinical visit, drugs and laboratory test or indirectly as loses of employment. Study indicates that healthcare expenditures on diabetes accounts for about 11.6% of the total healthcare expenditure in the world in 2010 (Seuring *et al.,* 2015). An ageing of global population, urbanization, rising prevalence of obesity and sedentary lifestyles are considered as contributing factors for fast increment of diabetes (Alemu, 2015).

1.2.2. Classification and diagnosis of diabetes

Diabetes is heterogeneous type of disease(disorder of the body's metabolic system) and based on its pathophysiology, clinical presentation, disease progression and laboratory finding classified into type one diabetes melitus (T1DM), T2DM, GDM (gestetional diabetes mellitus) and other specific type of diabetes like monogenic diabetes syndromes, diseases of the exocrine pancreas and drug- or chemical-induced diabetes. This classification is very important for determining therapy; drugs or lifestyle modification (American Diabetes Association, 2017; George *et al***.,** 2017). But many of diabetic individuals do not easily fit into a single class.

Diabetes may be diagnosed based on HbA1C criteria or plasma glucose criteria, either the fasting plasma glucose (FPG) or the 2-h plasma glucose (2-h PG) value after a 75-g oral glucose

tolerance test (OGTT). Standards for the diagnosis of diabetes includes HbA1C ≥ 6.5%, FPG ≥ 126 mg/dL (7.0 mmol/L), 2-h PG (OGTT) ≥ 200 mg/dL (11.1 mmol/L). HbA1C has several advantages to the FPG and OGTT, including greater convenience (fasting not required), greater preanalytical stability, and less day-to-day perturbations during stress and illness (American Diabetes Association, 2016). HbA1c also acts as a biomarker for dyslipidaemia as well as a potential indirect predictor of CVD risk in T2DM patients. The severity of the metabolic abnormality can progress, regress, or stay the same. Thus, the degree of hyperglycemia reflects the severity of the underlying metabolic abnormalities and its treatment outcome (Hussian *et al*.**,** 2017**;** Purohit *et al*.**,** 2017**;** Thambiah *et al*., 2016).

1.2.2.1. Type 1 diabetes mellitus (T1DM)

T1DM could be refered to as immune-mediated diabetes and it accounts about 5–10% of all diabetes case worldwide. Mostly, T1DM resulted from cellular-mediated autoimmune destruction of the ß-cells of the pancreas. Autoantibodies to islet cell, insulin, glutamic acid decarboxylase (GAD65), and to the tyrosine phosphatases IA-2 and IA-2ß are markers for T1DM; usually one or more of these autoantibodies are present in 85–90% of individuals when fasting hyperglycemia is initially detected. The rate of ß-cell destruction is quite variable, being rapid in some individuals (mainly infants and children) and slow in others (mainly adults). There is also idiopathic insulinopenia. Some patients, particularly children and adolescents, may present with ketoacidosis as the first manifestation of the disease. Mostly, T1DM patients are dependent on life long insulin therapy. These patients are also prone to other autoimmune disorders such as Graves' disease, Hashimoto's thyroiditis, Addison's disease, vitiligo, celiac sprue, autoimmune hepatitis, myasthenia gravis, and pernicious anemia (American Diabetes Association, 2017; Lucaccioni & Iughetti, 2016; Diaz-Valencia *et al*., 2015; Jin and She, 2012; Guariguata 2011).

1.2.2.2. Type 2 diabetes mellitus (T2DM)

T2DM is a chronic metabolic condition initially involving 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. About 90% of all newly diagnosed cases diabetes are T2DM. 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 (Kahn *et al*., 2014 Rotella *et al.*, 2013).

The major risk factors for T2DM are the following: age greater than 45 years, 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 < 35 mg/dl (0.9mmol/l) or triglyceride level > 250 mg/dl (2.82mmol/l), history of gestational diabetes mellitus or of delivering a baby with a birth weight of > 9lb. It is associated with long-term microvascular and macrovascular complications, together with reduced quality and expectancy of life (Alberti *et al.*, 2007, American Diabetes Association, 2017).

1.2.2.3. Gestational diabetes mellitus (GDM)

Gestational diabetes is carbohydrate intolerance resulting in hyperglycaemia of variable severity with onset or first recognition during pregnancy. Individuals at high risk for gestational diabetes include older women, those with previous history of glucose intolerance, women from certain high-risk ethnic groups etc. Usually GDM appears between 24 and 28 weeks of gestation and after the pregnancy ends, the woman could be re-classified as having either diabetes mellitus, impired glucose tolerance (IGT) or normal glucose tolerance based on the results of a 75 g OGTT done after six weeks or more after delivery (Leng *et al.*, 2015). The trend toward older maternal age, higher body mass index, abortions and parity, history of macrosomia, the epidemic of obesity and diabetes, the decrease in physical activity and lifestyles in developing countries may all contribute to an increase in the prevalence of GDM (Jafari-Shobeiri, *et al*., 2015; Ferrara, 2007).

1.2.3. Complication of diabetes

The prevalence of diabetes (DM) is constantly increasing worldwide at an alarming rate. According to the International Diabetes Federation report in 2015, over 415 million people globally were suffering from diabetes. Complications of DM account for increased morbidity, disability, and mortality and represent a threat for the economies of all countries, especially the developing ones. The underlying mechanisms in the pathogenesis of diabetic complications include certain genetic and epigenetic modifications, nutritional factors, and sedentary lifestyle (Papatheodorou *et al.*, 2016).

People with diabetes have an increased risk of developing a number of serious health problems. Consistently high blood glucose levels can lead to serious diseases affecting the heart and blood vessels, eyes, kidneys, nerves and teeth. In addition, people with diabetes also have a higher risk of developing infections. In almost all high-income countries, diabetes is a leading cause

of cardiovascular disease, blindness, kidney failure, and lower limb amputation. Diabetic complications can be classified as (a) microvascular complications which includes diabetic nephropathy, neuropathy, and retinopathy mainly induced by chronic hyperglycemia. (b) Macrovascular Complications (coronary artery disease, peripheral arterial disease, and stroke) and (c) Miscellaneous non vascular Complications. Diabetic cardiomyopathy which is a specific complication that develops independently of coronary artery disease or hypertension and it is possible to lead to increased morbidity and mortality (Jia *et al.*, 2016; Fowler, 2008)

1.2.4. Dyslipidemia in type 2 diabetes

Dyslipidemia is one of the major risk factors for cardiovascular disease in T2DM and it is highly prevalent (72–85%). The characteristic features of diabetic dyslipidemia are a high plasma triglyceride concentration, low HDL cholesterol concentration and increased concentration of small dense LDL-cholesterol particles (Kansal & Kamble, 2016; Thambiah *et al*., 2016; Mooradian, 2009). These changes are caused by increased free fatty acid flux secondary to insulin resistance and aggravated by increased inflammatory adipokines (Ouchi *et al*., 2011). The availability of several lipid-lowering drugs and nutritional supplements offers novel and effective options for achieving target lipid levels in people with diabetes. While initiation of drug therapy based on differences in the lipid profile is an option, most practice guidelines recommend statins as first-line therapy (Chehade *et al.*, 2013; Nakajima, 2010).

Diabetic dyslipidemia is caused by impaired action of lipoprotein lipase (LPL) that is localized to the endothelial cells. Smaller size and modified low-density lipoprotein (LDL), such as glycated and oxidized LDL, play important roles to induce vascular and renal cellular dysfunction through Rho-kinase activation. Rho-kinase plays a key role in the pathogenesis of Diabetic nephropathy by activating the inflammatory pathway, including oxidative stress, nuclear factor-κB (NF-κB), and hypoxia inducible factor (HIF)-1 and statin can inhibit Rho-kinase (Kawanami *et al.,* 2016; Vergès, 2015; Tenenbaum *et al.,* 2008;).

1.2.5. Most commonly available lipid lowering drugs

(a) Statins: Statins, also known as HMG-CoA reductase inhibitors, are a class of lipid-lowering medications in high CVD risk patients like T2DM. In most cases, treatment with statins continues for life, as stopping the medication causes blood cholesterol to return to a high level within a few weeks (Elnaem *et al.*, 2017; Lambert, 2014). Available statin via prescription in the UK are: atorvastatin (Lipitor), fluvastatin (Lescol), pravastatin (Lipostatin), rosuvastatin (Crestor) and simvastatin (Zocor). Higher doses of statins are more effective mainly for the

prevention of the nonfatal cardiovascular events but such doses are associated with an increase in hepatotoxicity, myopathy and leading to non-cardiovascular death. Statins may be less effective in reducing LDL cholesterol in people with familial hypercholesterolemia, especially those with homozygous deficiencies. These people have defects usually in either the LDL receptor or apolipoprotein B genes, both of which are responsible for LDL clearance from the blood (Pedro-Botet *et al.*, 2016; Stancu & Sima, 2001). Statin treatment is lifelong as withdrawal reverts the problem and a number of studies support the idea (Takata *et al*, 2017, Ford *et al*., 2016, Lu *et al*., 2016).

All statins makes the liver as target organ as for antidyslipidemic effect and metabolism. Antidyslipidemic effect could increase the activity of LDL receptor in hepatocytes there by decrease plasma LDL cholesterol level or inhibit HMG-CoA reductase, the enzyme that converts HMG-CoA into mevalonic acid. Statins also has anticoagulation, vasodilatation, antioxidant properties and anti-atherosclerotic effect (reduction of plaque stabilization, platelet activation, plaque proliferation and inflammation) (Trentman *et al.*, 2016; Stancu and Sima, 2001).

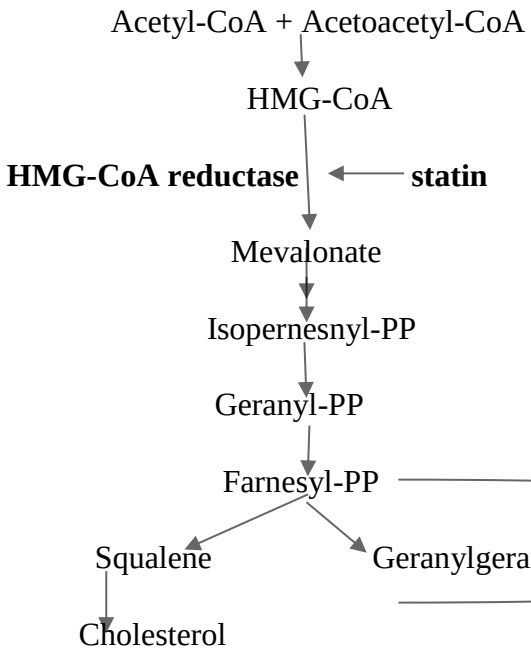


Figure 1: the pharmacological effect of statin on the mevalonate pathway.

Statins inhibit conversion of HMG-CoA to mevalonate by competitive inhibition of the rate limiting enzyme HMG-CoA reductase. Statins also inhibit the biosynthesis of several intermediates of the mevalonate pathway (Bonetti *et al*., 2003).

(b) Fibrates: Fibrates are weak agonists of peroxisomal proliferator activated receptor-alpha PPAR-α, a nuclear transcription factor thereby, inducing transactivation or trans repression of multiple genes, regulates fatty acid and lipoprotein synthesis; catabolism and several aspects of vascular wall biology (Azhar, 2010). Fibric acid derivatives (fibrates) are a class of medication that lowers blood triglyceride levels through reductions in acetyl-CoA carboxylase and fatty

acid synthase activity, inhibiting the esterification of diacylglycerols and it promotes the β-oxidation of fatty acids. Finally it reduces the liver's production of VLDL (the triglyceride-carrying particle that circulates in the blood) and by speeding up the removal of triglycerides from the blood. Fibrates are also modestly effective in increasing blood HDL cholesterol levels; however, they are not effective in lowering LDL cholesterol (Keating and Croom, 2007). The side effects of fibrates include nausea, stomach upset, sometimes diarrhea and liver inflammation. When fibrates used for several years can cause gallstones. In addition fibrates can cause muscle damage particularly when taken together with statin medications (Sharma *et al.*, 2015).

(c) PCSK9 (proprotein convertase subtilisin/kexin type 9) inhibitors: PCSK9 is an enzyme (serine protease) produced predominantly within hepatocytes, serves to regulate low-density lipoprotein receptor (LDL-R) density on hepatocytes. Mutations in the PCSK9 gene can cause either familial hypercholesterolemia or low concentrations of plasma LDL cholesterol in a subset of patients (Page and Watts. 2016). Proprotein convertase subtilisin/kexin type 9 inhibitors serve as lipid-lowering agents and have promising potential in threating dyslipidemia. By inhibiting the proprotein convertase subtilisin/kexin type 9 enzyme, this novel molecule leads to increased low-density lipoprotein receptor density and decreased circulation of LDL. As PCSK9 inhibitor is a monoclonal antibody, it has limited drug interactions and minimum adverse drug effect (Noel and Beavers**,** 2017).

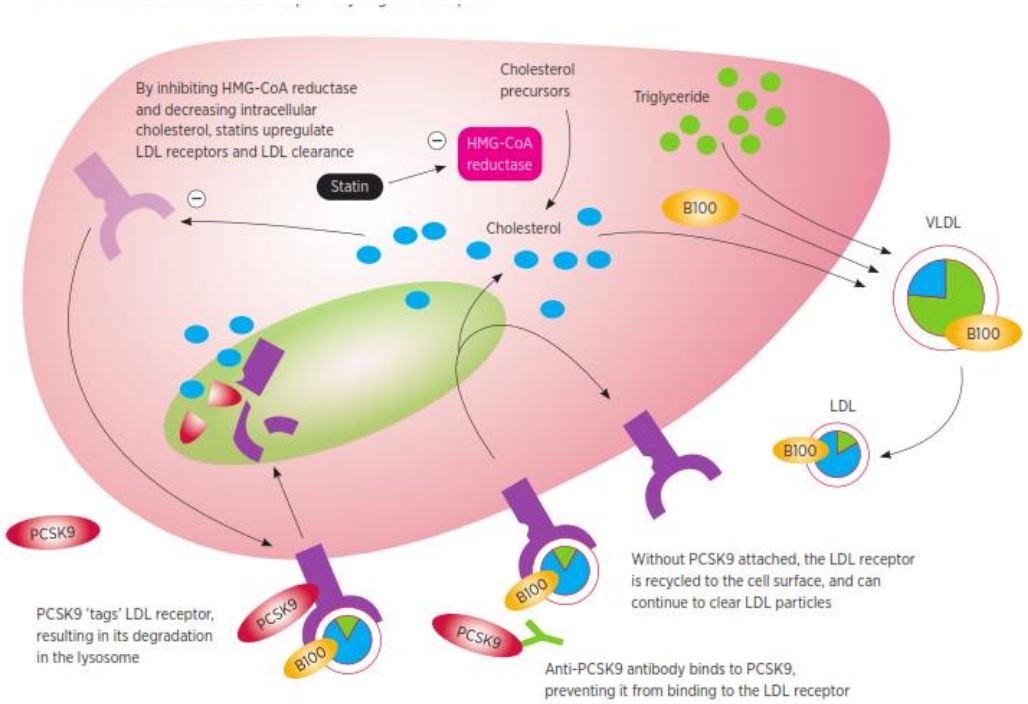


Figure 2.Mechanism of action of statin and anti-PCSK9 monoclonal antibodies.

LDL particles are taken up via LDL receptors, primarily on hepatocytes, and degraded. The production of LDL receptors is decreased by intracellular cholesterol, so lowering intracellular cholesterol

(**d) Ezetimibe:** the first selective cholesterol absorption inhibitor, offers an alternative therapy to those patients intolerant of statins or add-on therapy when targets are not reached. This is of relevance to those with increased vascular risk, including patients with diabetes. Ezetimibe targets uptake of cholesterol at the jejunal enterocyte brush border. Its primary target of action is the cholesterol transport protein Nieman Pick C1 like protein. Ezetimibe is an effective LDL-C lowering agent and is safe and well tolerated. There is significant controversy surrounding the use and therapeutic effectiveness of this drug. Humans ingest an average of 300mg of cholesterol per day and an additional 1g of cholesterol is secreted into the gut as bile and ezetimibe acts on exogenous cholesterol and bile. Its half-life of 22 hours allows the drug to be prescribed once daily (Nelson *et al.*, 2008; Phan *et al.*, 2012).

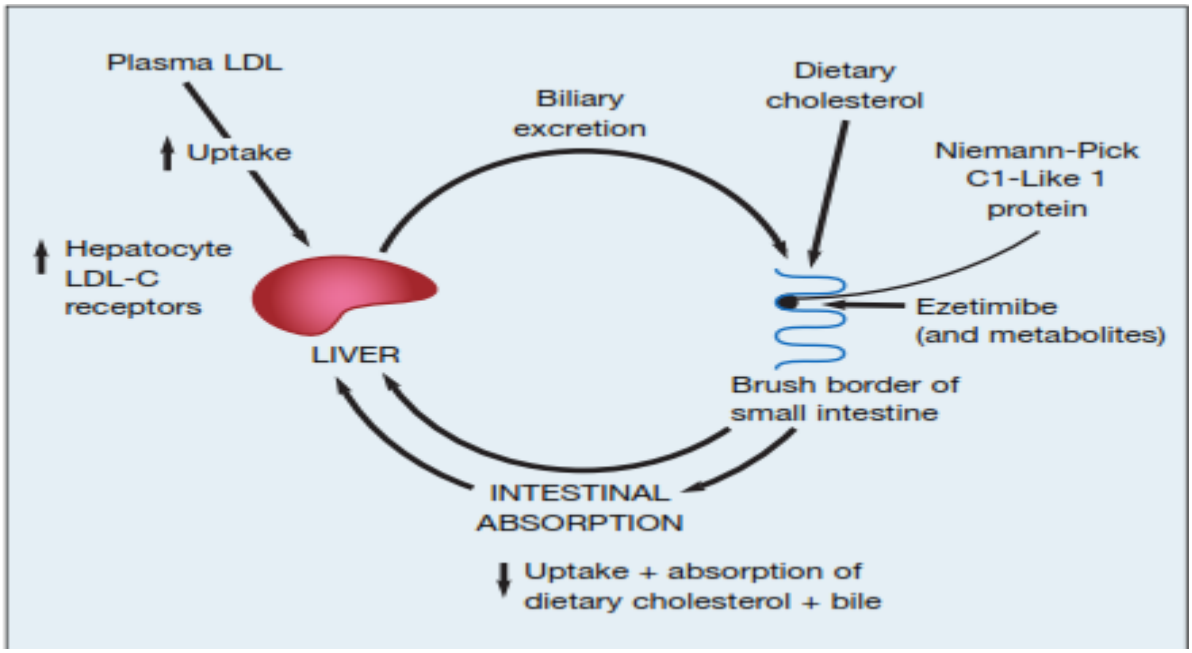


Figure 3. Pharmacological action of ezetimibe.

Plasma cholesterol concentration is dependent upon the balance between endogenous and exogenous cholesterol metabolism. Ezetimibe has a unique mode of action by targeting the exogenous pathway (Nelson *et al.*, 2008).

1.2.6. Drug induced hepatotoxicity

Drug-induced hepatic injury is the most frequent reason cited for the withdrawal of an approved drug from the market, and it also accounts for more than 50 percent of the cases of acute liver failure in the United States. The liver, located between the absorptive surface of the gastrointestinal tract and drug targets throughout the body, is central to the metabolism of virtually every foreign substance. Most drugs and xenobiotics are lipophilic, enabling them to cross the membranes of intestinal cells. Drugs are rendered more hydrophilic by biochemical processes in the hepatocyte, yielding water-soluble products that are excreted in urine or bile. This hepatic biotransformation involves oxidative pathways, primarily by way of the cytochrome P-450 enzyme system. After further metabolic steps, which usually include conjugation to a glucuronide or a sulfate or glutathione, the hydrophilic product is exported into plasma or bile by transport proteins located on the hepatocyte membrane, and it is subsequently excreted by the kidney or the gastrointestinal tract (Han *et al.*, 2017; Kullak-Ublick *et al*., 2017; Marrone *et al*., 2017).

Most drugs cause liver injury infrequently. These reactions are considered idiosyncratic, occurring at therapeutic doses from 1 in every 1000 patients to 1 in every 100,000 patients, with

a pattern that is consistent for each drug and for each drug class. Idiosyncratic reactions are characterized by a variable delay or latency period, ranging from 5 to 90 days from the initial ingestion of the drug, and are frequently fatal if the drug is continued once the reaction has begun (Björnsson, 2016; Smilkstein *et al.,* 2003).

Idiosyncratic drug-induced liver injury pathogenesis is complex, depending on the interaction of drug physicochemical properties and host factors (age, sex, race, underlying liver disease etc). Regardless of stringent requirements for drug development imposed by regulatory agencies, drug-induced liver injury is an increasing health problem and a significant cause for failure to approve drugs, market withdrawal of commercialized medications and adoption of regulatory measures. Drug-induced liver injury has a very broad spectrum of presentation ranging from asymptomatic elevations of liver aminotransferases to acute liver failure and liver Biochemistry test results are used to define liver injury early. According to international consensus; (a) alanin-aminotransferase (ALT) ≥5x upper limit of normal (ULN); (b) alkaline phosphatase (ALP) ≥2x ULN or (c) ALT ≥3x ULN and total bilirubin (TB) ≥2x ULN are indicators of drug induced liver injury. Pattern of drug induced liver injury could be one of the following: acute hepatitis, chronic hepatitis, acute cholestasis, chronic cholestasis and cholestatic hepatitis (Ortega-Alonso *et al.,* 2016). Drug induced acute and chronic cholestatic liver injury can present with asymptomatic disease where the only clinical manifestation is an elevation in alkaline phosphatase. Most cases of drug induced cholestasis and hepatitis will resolve with withdrawal of the offending medication (Padda *et al*., 2011).

1.2.6.1. Statin induced hepatic injury

Its cholesterol and non-cholesterol (pleiotropic) effects put statins as an efficacious drug in the prevention of cardiovascular events associated with increased blood lipids and atherosclerotic lesions (O'Sullivan, 2007). Diabetic patients are considered as high risk patients for CVD as a result, all diabetes age greater than 40 years old are recommended to start statin therapy, but study indicate only half of them are on therapy (Mortensen *et al.,* 2016). According to National Institute for Health and Care Excellence [NICE] recommendation, in United Kingdom primary prophylaxis with atorvastatin should now be considered for all individuals between the age of 30 to 84 years with a 10 year cardiovascular risk score of 10% or greater (Rabar *et al.,* 2014). This guidance has put controversy over possible adverse effects of statins in a healthy population. Statins are widely used antidyslipidemic drug and generally well tolerated. Study indicates there is an incidence of serum transaminases elevation (Clarke *et al.,* 2016; Teschke,

2012). Laboratory evaluation of patients who are on intensive atorvastatin therapy for 8 months revealed much increased levels of aminotransferases (Dragos *et al*., 2017). As data indicates there were elevation of serum transaminases of hepatic origin in about 1–3% of individuals treated with statins, but occasionally more severe reactions can occur with possible autoimmune hepatitis, resulting in hospitalization and deaths. Clinical trials have shown that statin use has been associated with elevations in serum alanine aminotransferase (ALT) levels in approximately 3% of persons who were taking the drugs. There was also observed hepatocellular carcinoma in association with statin use (Thapar *et al.,* 2013). Study done in China indicates ALT >1 × ULN, was higher in statins group than that in no statins group. And proportion of elevated ALT in patients with stain use < 1 month was higher as compared to those with stain use ≥3 months (Gao *et al.,* 2017). But most studies looking at drug-induced liver disease related to statins have relied on results from registration of clinical studies or self-reported adverse drug events which may lead to under-reporting of cases (Björnsson *et al.*, 2012).

1.2.7. Drug induced muscle injury

Drug-induced muscle injury is a common syndrome that is complex and potentially life threatening. Rhabdomyolysis is defined as skeletal muscle injury that leads to the lysis of muscle cells and the leakage of myocyte contents into the extracellular compartments. The presenting clinical features are myalgias, myoglobinuria, and an elevated serum creatine kinase. The etiology of skeletal muscle injury is quite diverse, including excessive muscular stress and ischemia, genetic defects, and direct toxic or physical damage; drugs and alcohol have become frequent causative agents in up to 81% of cases of rhabdomyolysis. Drug-induced rhabdomyolysis can be a primary direct insult on the skeletal myocyte function and integrity or secondary effects of toxins due to predisposing risk factors such as local muscle compression in coma, prolonged seizures, trauma, and metabolic abnormalities. But most drug-induced myopathies are potentially reversible if recognized early (Holder, 2016).

Creatine kinase (CK) also known as creatine phosphokinase (CPK) or phosphocreatine kinase is an enzyme expressed by various tissue and cell type. The function of CK in biological system is to convert myocyte creatine phosphate into high energy phosphate groups (adenosine triphosphate) used in energy requiring reactions. CK activity is typically high in skeletal muscle type II fibers (Oudman, 2013). In biological system, CK catalyze the following reaction

Creatine +ATP $\xleftrightarrow{\text{creatine kinase}}$ phosphocreatine + ADP

Degradation of approximately 200 g of muscle can cause an increase in serum CK. Therefore, total serum CK is the most sensitive biochemical indicator of rhabdomyolysis or muscle injury (Coco and Klasner, 2004). There is also drug induced myocardial injury defined as the disruption of normal cardiac myocyte membrane integrity leading to release of intracellular components including structural proteins such as troponin (Tn) and creatine kinase-MB (CK-MB) isotype. Drugs like statin has increased serum CK over 100x from ULN and it is consistent with degree of muscle injury (Egholm and Pareek, 2015).

1.2.7.1. Statin induced muscle injuries

Studies have shown that statins are safe with regard to muscle; study done by Parker *et al.* (2013) to measure CK, exercise capacity, and muscle strength before and after atorvastatin 80 mg or placebo was administered for 6 months on 420 healthy, statin-naive subjects indicated no individual CK value ≥10ˣ ULN, but average CK increased. Other similar study done by Soko *et al*. (2016) in Zimbabwean didn't show any serious adverse effect of statin on muscle. The need for statin treatment for both primary and secondary prevention of cardiovascular events is widely recognized in almost all diabetic patients. The use of statin treatment for primary prevention without risk-elevation is however, more controversial (de Vries, 2016; Stroes *et al.,* 2015). Many reviews indicate that statin-associated myopathy, with significant elevation of serum CK, is a rare but serious side effect of statins, affecting 1 per 1000 to 1 per 10 000 people on standard statin doses. The condition cover a broader range of clinical presentations range from myalgia, myositis, and rhabdomyolysis to asymptomatic increase in the blood levels of CK (Stroes *et al.,* 2015). Suggested theories about mechanism of statin induced myopathy: impaired synthesis of cholesterol for myocyte membranes, impaired synthesis of coenzyme Q10 (ubiquinone) for mitochondrial function and depletion of isoprenoids (increase myofibril apoptosis) and altered muscle protein degradation. Individual drug responses can also be affected by genetic and environmental factors (Ambapkar *et al.,* 2016). Statin induced muscle toxicity increase with dose (Auer *et al.,* 2016) and duration of time being on therapy (Calza *et al*., 2017).

Lactate dehydrogenase (LDH), a tetrameric intracellular enzyme with two basic subunits, is found in most of major organ tissues and catalyze the conversion of lactate to pyruvate (forward) and pyruvate to lactate (reverse) reaction with concomitant oxidation and reduction of nicotine amide dinucleotide (NAD). Serum levels of LDH are elevated in a wide variety of pathologic conditions, most notably cardiac, hepatic, and other hematologic changes

(Mohammed & Elias, 2016; Huijgen *et al*., 1997). In association with hepatic and muscular injury induced by statin, plasma level of LDH may be elevated and act as a biomarker (Noor *et al.,* 2016; Tokinaga *et al.,* 2006). Intensive (high dose) statin therapy could exaggerates its adverse effect (Dragos *et al*., 2017, Unnikrishnan and Satish, 2005).

1.3. STATEMENT OF THE PROBLEM

T2DM (due to a progressive loss of ß-cell insulin secretion frequently on the background of insulin resistance) is present in the range of 85-95% of all diabetic cases in high-income countries. According to the latest data from WHO, 422 million adults are living with diabetes mellitus and it is recognized as an important cause of death and disability worldwide. In 2012, there were 1.5 million deaths worldwide directly caused by diabetes (World Health Organization, 2016). Incidence assessment study done in different parts of Ethiopia showed DM is getting increased in an alarming rate and become great issue of Ethiopian health system (Abebe *et al.*, 2013).

Individuals with T2DM have a higher incidence of dyslipidemia which is characterized by elevations of serum TAG, LDL cholesterol and decreased in HDL cholesterol values than non-diabetes. Many studies revealed that dyslipidemia is associated with DM. As a result, patients with T2DM has high risk of developing cardiovascular diseases and are advised to be treated for dyslipidemia (Bali & Vij, 2016; Borle *et al.*, 2016)). Since individuals with T2DM are considered as high risk for atherosclerotic cardiovascular disease (ASCVD), almost all T2DM patients above age 40 years are advised to take lipid lowering drugs mainly statins (Balgi *et al.*, 2017; Rutishauser, 2006) .

Statins are considered very effective in reducing cardiovascular morbidity and mortality in high-risk patients and its safety was well established. But several clinical studies have demonstrated evidences of liver toxicity and statin-associated myopathy. Studies indicate that genetic and environmental factors determine statin induced liver and muscle toxicity and its effectiveness as well (Stroes *et al.*, 2015; Rosenson *et al.*, 2014; Bitztur *et al.*, 2013). In Ethiopia, there is no well documented study done to examine the extent of compliance and adverse reaction to progression of liver function tests, serum CK and LDH level among T2DM patients who are receiving lipid lowering drugs like statin. The present study was done to assess the causative link of liver and muscle function abnormality progression at different time periods among T2DM patients who are receiving statin therapy at TASH.

1.4. SIGNIFICANCE OF THE STUDY

Diabetes Mellitus is a common incurable chronic disease. T2DM is highly prevalent form of diabetes throughout the world and the disease reach to epidemic level in low and middle income countries, very common in Ethiopia. Dyslipidemia which is the main risk factor for cardiovascular disease development is one of the great metabolic disorder among diabetes patients mainly in T2DM patients. As a result, T2DM patients are considered as high risk group for ASCVD and advised to start lipid lowering drugs mainly statins.

According to International Diabetes Federation Africa report, there were over 1.33 million cases of diabetes in Ethiopia in 2015 and by 2040 this figure will be more than double. Majority of diabetic cases are T2DM (85-90%) which are very important contributing factors for dyslipidemia.

Previously published works indicate that hepatotoxicity characterized by plasma elevation of liver enzymes and myopathies are the most common patient compliance reported and case for non-adherence of drug among patients who are receiving statin. Different host, environmental factors and time length of drug use can affect the drug interaction and they are important information for patient safety. Assessment of drug induced organ failure has indispensable advantage for patient. For that reason, the present study is attempt to provide a comparative explanation of liver function tests, CK and LDH assay among T2DM patients who were on statin at different time periods visiting TASH. It is very useful to provide a base line information for other researchers conducting study in related idea.

1.5 HYPOTHESIS OF THE STUDY

HO: Means of serum lipid profile, liver enzymes, CK-MB isotype and LDH among T2DM patients who were on statin drugs at different time periods would be similar

HA: means of serum lipid profile, liver enzymes, CK-MB isotype and LDH among T2DM patients who were on statin drugs at different time periods would be significantly changed.

CHAPTER- TWO

OBJECTIVE

2.1. General Objective: To compare the level of lipid profile, liver enzymes, CK-MB & LDH of T2DM patients who were on statin therapy.

2.2. Specific Objectives

- To assess the level of serum lipid profile, liver enzymes, CK and LDH in T2DM patients who were on statin therapy
- To evaluate the level of serum lipid profile, liver enzymes, CK and LDH in T2DM patients who were not on statin therapy.
- To compare the level of lipid profile, liver enzymes CK and LDH among T2DM patients who were on statin therapy with control

CHAPTER- THREE

METHOD AND MATERIALS

3.1. Study Area and Period

This study was conducted at Diabetic Clinic of Tikur Anbessa Specialized Hospital (TASH). TASH is a large referral teaching hospital, under the administration of Addis Ababa University, located in Lideta Sub City Addis Ababa, Ethiopia. This referral hospital has more than 700 beds for patients and is the main teaching hospital for both clinical and preclinical training of most disciplines. Patients are referred to this hospital from other health institution across the country. It is also an institution where specialized clinical services that are not available in other public or private institutions are rendered to the whole nation. The hospital offers diagnosis and treatment for more than 400,000 patients per a year.

In TASH, there are different units and clinics that provide specialized service for patients. Among these, diabetic center is the one which provide service for patients with different endocrinological problem. The reason for selecting TASH for this study is that there is center only for diabetic patients. Hence, the study participants from all over Ethiopia visits this center. This saves time and cost of the study.

The study was conducted from March 2017 to September, 2017. Blood analysis was conducted at Zewditu Memorial Hospital and at Ethiopian Public Health Institution clinical chemistry laboratory unit.

3.2. Study Design

Hospital based cross-sectional study design was used to determine serum level of liver enzymes (ALT, AST and ALP), CK-MB isotype, LDH and lipid profiles(LDL, HDL,TAG and total cholesterol) among T2DM patients who were receiving statin and not receiving statin at Diabetic Clinic of TASH within the study period.

3.3. Population

3.3.1. Source Population

The source population for this study was all diabetic patients attending Diabetic Clinic of TASH.

3.3.2. Study Population

The study population for this study was all T2DM patients who were receiving statin drugs attending at Diabetic Clinic of TASH during the study period and who were not on statin therapy as control group.

3.4. Sampling Technique

To recruit study subjects convenience sampling method was applied**.**

3.5. Sample Size Determination

The sample size of the study was being calculated based on single population formula as follows:

$$n = (Z_{1-\alpha/2})^2 P (1-P)/d^2$$

n = 45

10% non-respondent = 5, total sample size of the study: n =50 T2DM patients

Where: d = marginal error which is 5%

n = minimum sample size required for this study

p = Mild elevations in serum aminotransferases arise in up to 3% of statin treated patients elevation in different clinical trial. (Russo *et al*., 2014; Thapar *et al.*, 2013)

$Z_{1-\alpha/2}$= Confidence Interval (CI) at 95% which is 1.96

As the study intended to assess the level of lipid profile, liver enzymes, CK and LDH among T2DM who were on statin therapy at different time periods, it was done on a total of 100 T2DM patients divided in to four groups (Group I, n = 25 T2DM patients who were on statin for 14 days – 6 months, Group II, n = 25 T2DM patients who were on statin for 6 – 18monthes, Group III, n = 25 T2DM patients who were on statin for more than 18monthes and Group IV, n = 25 T2DM patients who were not on statin therapy) (Gao *et al.*, 2017, Ford *et al*., 2016, Joo, 2012;).

3.6. Study Variables

3.6.1. Dependent variables

- Liver enzyme level (AST, ALT, ALP)
- Serum CK-MB isotype
- Serum LDH

- Serum lipid profile

3.6.2. Independent variable

- Age, educational level, economic status
- Type of drugs
- Alcohol intake status
- Cigarette smoking condition
- Duration of statin use

3.7. Eligibility criteria

3.7.1. Inclusion criteria

- T2DM patients who were on statin drugs.
- T2DM patients who were not on statin drugs as control group attending at TASH were included in this study.

3.7.2. Exclusion criteria

Patients who had any clinical evidence of

- Cirrhosis or other causes of chronic liver disease
- Diagnosed T1DM
- Pregnancy and lactating
- Children less than 18 years of age
- Using HIV drugs
- Known muscular and cardiac disease were excluded from this study.

3.8. Methods of data collection and process

3.8.1. Socio-demographic

Data was collected by well trained Nurses and data collection form (questioner) was designed to record all valuable information (sex, age, educational status, economic status, dietary condition, and medical history of each patient).

3.8.2. Body Mass Index (BMI) and Waist-to-hip ratio (WHR)

Body Mass Index is a useful clinical calculation to diagnose obesity because it is correlated with total body fat. BMI has been calculated as weight (kg) divided by height in meter square (m^2). Values of BMI could be classified as follows. BMI ≤18.5 Underweight, BMI =18.5-24.9

normal weight, BMI=25-29.9 overweight and BMI ≥30 obese. Similarly, WHR is used as measurements of obesity which in turn is a possible indicator of other more serious health condition. According to WHO, WHR of above 0.9 for male and 0.8 for female is considered as abdominal obesity. Weight, height, waist and hip were measured by experienced nurse and BMI and WHR were calculated.

3.8.3. Blood sample collection and analysis

Five ml of venous blood was drawn from each volunteer T2DM patient using a disposable plastic syringe. The blood was poured into serum separator tube (SST) and then centrifuged after it was clotted. Serum was kept at -80°C in refrigerator till biochemical analysis was carried out. Liver enzymes (AST, ALT, ALP), CK-MB, LDH and lipid profile (LDL, HDL, TAG and total cholesterol) were measured with spectrophotometer by cobas c analyzer. Manufacturer instruction of reagent and instrument was strictly followed (Imai *et al.*, 2008).

3.8.4. Laboratory Testing Methods

A. Aspartate Aminotransferase (AST) assay method

AST is a cellular enzyme present in many tissues such as heart, skeletal muscles, kidney, brain, liver, pancreas or erythrocytes. It exists in two isoforms, cytoplasmic and mitochondrial. The cytoplasmic isoenzyme is released into the blood during the moderate cell damage. On the other hand, the activity of the mitochondrial isoenzyme in blood increases during the severe cell damage. The determination of AST activity in serum is used mainly to assess the liver damage. AST in biological system catalyzes the following reaction:

- Aspartate + α-ketoglutarate $\xleftrightarrow{\text{AST}}$ oxaloacetate + glutamate
- Oxaloacetate + NADH + H^+ $\xleftrightarrow{\text{malate dehydrogenase}}$ malate + NAD^+

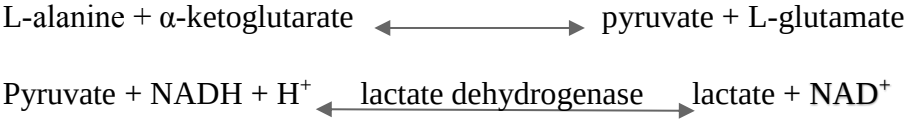
The reaction was monitored kinetically at 340 nm by the rate of decrease in absorbance resulting from the oxidation of NADH to NAD+ proportional to the activity of AST present in the sample. The method is linear up to 800 IU/L

Reference values: Normal value of AST for male and female at age greater than 20 years is 10-50u/l and 10-35 IU/L respectively (Ruhl and Everhart, 2012, Thapa and Walia, 2007).

B. Alanine Aminotransferase (ALT) assay methods

In the reaction, ALT catalyzes the reversible transamination of L-alanine and α-ketoglutarate to pyruvate and L-glutamate. The pyruvate is then reduced to lactate in the presence of lactate

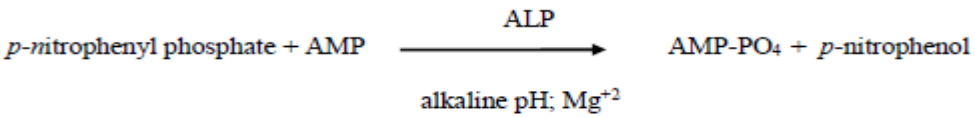
dehydrogenase (LDH) with the concurrent oxidation of NADH to NAD. The system monitors the rate of change in absorbance at 340 nm over a fixed time interval. The rate of change in absorbance is directly proportional to the ALT activity in the sample. ALT measurements are used in the diagnosis and treatment of liver and heart disease. The method is linear up to 800 IU/L.



Normal value of ALT for male and female at age greater than 20 years is 11-47 IU/L and 7-30 IU/L respectively (Ruhl and Everhart, 2012, Thapa and Walia, 2007).

C. Alkaline phosphates (ALP) assay methods

The method uses an enzymatic reaction rate using a 2-Amino-2-Methyl-1-Propanol (AMP) buffer to measure ALP activity in serum. ALP hydrolyses p-nitrophenyl phosphate and the phosphate is transferred to AMP. The increase in absorbance at 405 nm at 37 ^{0}C was measured and this was proportional to the amount of alkaline phosphatase that was present in the sample. The method is linear up to 1200 IU/L (Human gesellschaft for biochemical and diagnostic mbh – Germany). ALP measurements are used in the diagnosis and treatment of liver, bone, and parathyroid disease



Normal value of ALP for male and female at age greater than 20 years is 36-113IU/L (Ruhl and Everhart, 2012, Thapa and Walia, 2007).

D. Creatine kinase (CK-MB) assay methods

Creatine kinase (CK) appears as three isoenzymes which are dimers composed of two types of monomer subunits. The isoenzymes comprise all three combinations of monomers, M (for skeletal muscle derived) and B (for brain derived), as represented by the notations MM, MB, and BB. The CK-M subunits were inhibited by specific antibodies. Since CK-BB occurs rarely in serum it is assumed that the CK-B activity is derived from CK-MB present in the specimen. The activity of the CK-B subunits was determined and multiplied by two to provide an estimate of the CK-MB activity. The CK was activated by N-acetylcysteine (NAC). In a primary

reaction, the activated CK catalyzes the dephosphorylation of creatine phosphate to form creatine and ATP. In a coupled reaction catalyzed by hexokinase (HK), glucose was phosphorylated by ATP to form D-glucose-6-phosphate (G6P). Finally, glucose-6-phosphate dehydrogenase (G6PDH) catalyzed the oxidation of G6P by NADP+ to form 6-phosphogluconate and NADPH.

Creatine phosphate + ADP $\xrightarrow{\text{Creatine kinase}}$ creatine + ATD

ATP + glucose $\xrightarrow{\text{hexokinase}}$ glucose- 6- phosphate + ADP

Glucose-6-phosphate + NAD $\xrightarrow{\text{glucose-6-phosphate dehydrogenase}}$ gluconate-6-phosphate + NADH + H^+

The rate of the NADPH formation was directly proportional to the catalytic CK-MB activity. It was determined by measuring the increase in absorbance photometrical at 340 nm. Reference intervals strongly depend on the patient group regarded and the specific clinical situation. For healthy people: Reference range (37 °C) CK-MB < 25 U/L. For myocardial infarction diagnosis using the combination CK and CK-MB activity, and representing a CK consensus value based on long-term experience:

1. CK men > 190 IU/L (3.12 µkat/L)

CK women > 167 IU/L (2.87 µkat/L)

2. CK-MB > 24 IU/L (0.40 µkat/L)

3. The CK-MB activity accounts for 6-25 % of the total CK activity (Kiranmayi *et al.*, 2015).

E. Lactate dehydrogenase (LDH) assay methods

LDH is an enzyme which can be found in most major tissues. LDH assays can be performed by assessing LDH released into the media as a marker of dead cells or performing lysis LDH as a marker of remaining live cells. In this case LDH released into extra cellular fluid (plasma) as a marker of dead cells was performed. LDH catalyzes the conversion of lactate to pyruvate, the forward reaction and the conversion of pyruvate to lactate, the reverse reaction. This study utilized the forward reaction LDH assay method.

L-Lactate + NAD^+ $\xrightleftharpoons{\text{lactate dehydrogenase}}$ pyruvate + NADH + H^+

The initial rate of the NADH formation was directly proportional to the catalytic LDH activity and these was measured. It was determined by measuring the increase in absorbance. NADH strongly absorbs light at 340 nm, whereas NAD^+ does not.

Expected values: measured at 37 °C

Females 135-214 IU/L, Males 135-225 IU/L, Children (2-15 y) 120-300 IU/L and Newborns (4-20 d) 225-600 IU/L (Ekpe and Omotoso, 2015*).*

F. Lipid profile

Total cholesterol test principle: - Cholesterol was measured enzymatically in serum by using a series of coupled reactions that hydrolyze cholesteryl esters and oxidize the 3-OH group of cholesterol. One of the reaction byproducts, H_2O_2 was measured quantitatively in a peroxidase catalyzed reaction that produces a color. Absorbance was measured at 500 nm. The color intensity was proportional to cholesterol concentration.

The reaction sequence was as follows:

Cholesterol ester + H_2O $\xrightarrow{\text{cholesterol ester hydrolase}}$ cholestero + free fatty acid

Cholestero + O_2 $\xrightarrow{\text{cholesterol oxidase}}$ cholest -4en-3-one + H_2O_2

H_2O_2 + 4-aminophenazone + phenol $\xrightarrow{\text{peroxidase}}$ 4-(p-benzoquinone-monoimino)phenazone + H_2O

Cholesterol measurements are used in the diagnosis and treatment of atherosclerotic coronary artery disease and in the diagnosis of metabolic disorders involving lipids and lipoproteins metabolism. Desirable cholesterol levels are considered to be those below 200 mg/dl in adults and below 170 mg/dl in children.

LDL: - For this study direct LDL-cholesterol was measured by direct LDL assay principle which was takes place in two steps as follows.

1. Elimination of non-LDL lipoprotein

   Cholesterol ester + H_2O $\xrightarrow{\text{Cholesterol esterase}}$ Cholesterol + fatty acid

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

   $2H_2O_2$ $\xrightarrow{\text{catalase}}$ $2H_2O + O_2$

2. Measurement of LDL-C

   Cholesterol ester + H_2O $\xrightarrow{\text{Cholesterol esterase}}$ Cholesterol + fatty acid

   Cholesterol + O_2 $\xrightarrow{\text{cholesterol oxidase}}$ 4-cholestenone + H_2O_2

   $2H_2O_2$ + TOOS +4-AA $\xrightarrow{\text{peroxidase}}$ $4H_2O$ + quinonimine

The intensity of the color formed was directly proportional to the LDL-Cholesterol concentration in the sample.

HDL: - HDL was measured directly in serum. The apoB containing lipoproteins in the serum were reacted with a blocking reagent that renders them non-reactive with the enzymatic cholesterol reagent under conditions of the assay. The apoB containing lipoproteins were thus effectively excluded from the assay and only HDL-cholesterol was detected under the assay conditions.

Normal healthy person has 40-60mg/dl of serum HDL. The reaction principle was as follows.

1. ApoB containing lipoprotein + α-cyclodextrin + Mg+2 → soluble non-reactive complex with apoB-containing lipoprotein

2. HDL-cholesterol esters $\xrightarrow{\text{PEG- cholesterol esterase}}$ HDL-unesterified cholesterol + fatty acid

3. Unesterified + O_2 $\xrightarrow{\text{PEG- cholesterol oxidase}}$ cholestenone + H_2O_2

4. H_2O_2 + 5-aminophenazone + N-ethyl-N-(3-methylphenyl)-N - succinyl ethylene diame $\xrightarrow{\text{peroxidase}}$ H_2O + H+ + qunoneimine dye

TAG: - Triglycerides was measured enzymatically in serum using a series of coupled reactions in which triglycerides was hydrolyzed to produce glycerol. Glycerol was then oxidized using glycerol oxidase, and H_2O_2, one of the reaction products, was measured as described above for cholesterol. Absorbance was measured at 500 nm.

Triglycerides + $3H_2O$ $\xrightarrow{\text{lipase}}$ glycerol + fatty acids

Glycerol + ATP $\xrightarrow{\text{glycerokinase}}$ glycerol-3-phosphate + ADP

Glycerol-3-phosphate + O2 $\xrightarrow{\text{glycerolphosphate oxidase}}$ dihydroxyacetone phosphate + H2O2

H2O2 + 4- aminophenazone + 4-chlorophenol $\xrightarrow{\text{peroxidase}}$ 4-(p-benzoqunione-monoimino-phenazone + $2H_2O$ + HCL

Assessment of serum triglycerides help mark conditions that are associated with increased risk for CHD and peripheral atherosclerosis.

Desirable fasting triglyceride levels are considered to be those below 200 mg/dl, and are further categorized as Borderline, 200-400 mg/dl; high, 400-1,000 mg/dl; and very high (> 1000 mg/dl) (Kwiterovich, 2003-2004).

Blood glucose determination:- blood glucose analysis is ordered to measure the amount of glucose in the blood at the time of blood sample collection. It is used to detect both

hyperglycemia and hypoglycemia for diagnosis of diabetes. Glucose oxidase is highly specific for glucose and does not react with other saccharides. Glucose oxidase catalyze the oxidation of ß-D- glucose present in the plasma to D-glucono-1, 5-lactone with the formation of H_2O_2. The lactone then slowly hydrolyzed to D-gluconic acid. H_2O_2 broken down by peroxidase in the presence of oxygen and ortho toluidine which was converted to colored compound and the amount was measured colorimetric ally.

3.9. Data Analysis

Data entry and analysis was carried out using Statistical package for social science version 22. Descriptive statistics like frequency, proportion, mean, median and standard deviation was employed to describe socio demographic, clinical and behavioral characteristics of patients. One way ANOVA test was used to assess presence of significant difference in the means of lipid profile, liver enzymes, CK-MB and LDH among the study groups. Variables that were found significant at p-value <0.05 in one way ANOVA was included in to post hoc Turkey multiple comparison test. Statistical significance was declared at $p< 0.05$.

3.10. Ethical consideration

Ethical clearance was obtained from Research and Ethical Committee of the Department of Biochemistry, School of Medicine, and College of Health Sciences with protocol number: M.Sc. 8/17. Consent form was prepared with detailed explanation of objectives, risks, benefits to the study participants and the confidentiality was presented to the participant and kept. Data was collected after obtaining informed consent and agreement from the patients under study. Sample collection was performed by trained health professionals following ethical steps and scientific procedures.

3.11. Quality Assurance

The data quality assessment was started with socio-demographic data collection and have gone through blood sample collection, laboratory test and final data entry and statistical analysis. The blood sample was taken under aseptic techniques with standard operational procedure. The machine for biochemical analysis was checked for its consistency. Results was checked for completeness on daily basis by the immediate supervisor. Great attention in data insertion to software on computer was sought. The complete result was rechecked repeatedly to maintain the overall quality of data.

CHAPTER FOUR

Results

A total of 100 study participants in four groups; 25 T2DM who were on statin drug for 14days – 6months (group I), 25 T2DM who were on statin drug from 6months – 18months (group II), 25 T2DM who were on statin drug for greater than 18 months (group III) and 25 T2DM who were not on statin drug (group IV) were included in this study. All of T2DM patients participated in this study were on hypoglycemic drugs. Among the total participants, 48/100 (48%) were males and 52/100 (52%) were females and the numbers of male and female in each group were more or less similar. Mean age of the participant in group I, II, III and IV was 57 ± 7.77, 55.28 ± 7.51, 58.4 ± 9.36 and 52.9 ± 8.46 years respectively. The total (ground) mean age was 55.92 ± 8.69 years, ranging between 38 -74 years. About 89/100(89%) of the study participants have been educated up to preparatory and above and 11/100 (11%) were illiterate. Average monthly income of the study participants was 3144.12 ± 2320.31 Birr with range of 464 - 12000 Birr.

Table 1: Age sex distribution and average monthly income of T2DM patients who were on statin therapy at different time periods and T2DM patients who were not on statin at Diabetic clinic of TASH, Addis Ababa, 2017.

Variable	Group I (N=25)	Group II (N=25)	group III (N=25)	group IV (N=25)	Total (N=100)	*p-value*
Age mean ± SD Range	57 ± 7.8 43-69	55.3 ± 7.5 38-67	58.4 ± 9.4 41-74	52.9 ± 8.5 40-74	55.9±8.7 38-74	.119
Sex Male Female	12 13	13 12	10 15	13 12	48 52	.818
income mean ± SD RANGE	2667±1550 500-6900	3846±2940 630-12000	2250±1995 500-9000	3810±2246 464-12000	3144±2320 464-12000	.025

Group I= T2DM patients who were on statin for 14 days - 6 months, Group II= T2DM patients who were on statin for 6 -18 months, Group III= T2DM patients who were on statin for >18months, Group IV= T2DM patients who were not on statin. Significance was considered at $p < 0.05$.

Most of the participants (81%) in this study were living in Addis Ababa and the other 13/100(13%) were from Oromia region (fig. 4).

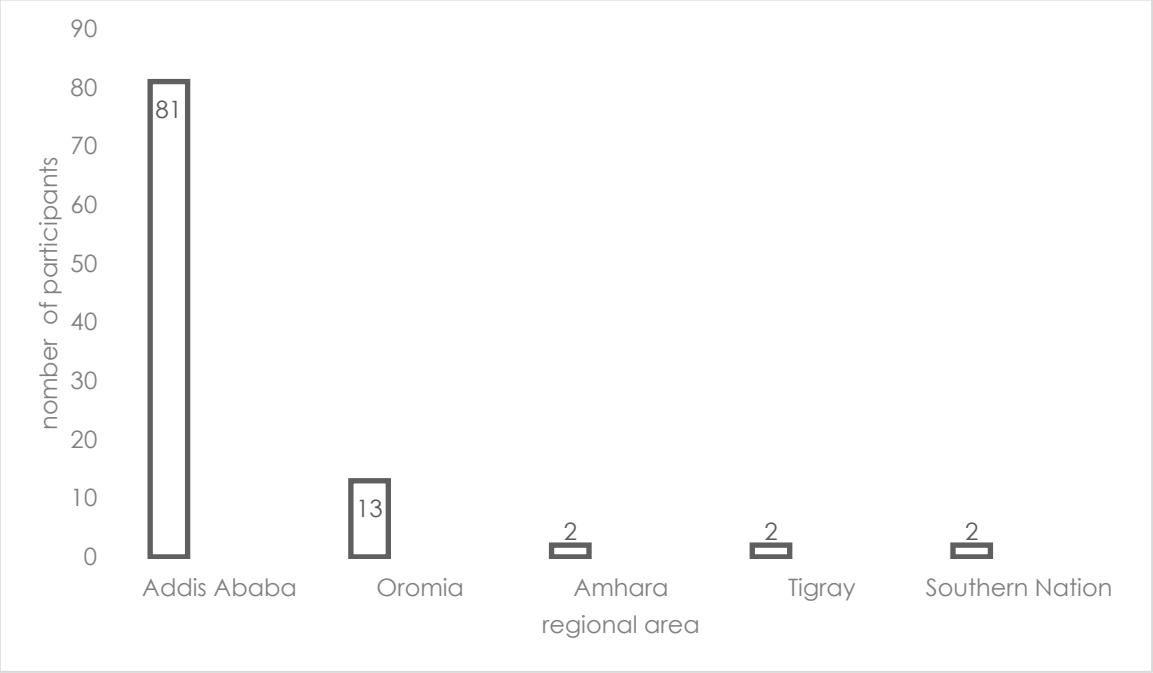


Figure 4: Reginal residence of the study participants at Diabetic Clinic of TASH, Addis Ababa, 2017

As shown in the fig. 5 below, 29/75 (38.67%), 28/75 (37.33%), 15/75 (20%) and 3/75 (4%) of the participants in this study used simvastatin, atorvastatin, lovastatin and fluvastatin respectively as anti-dyslipidemic drugs.

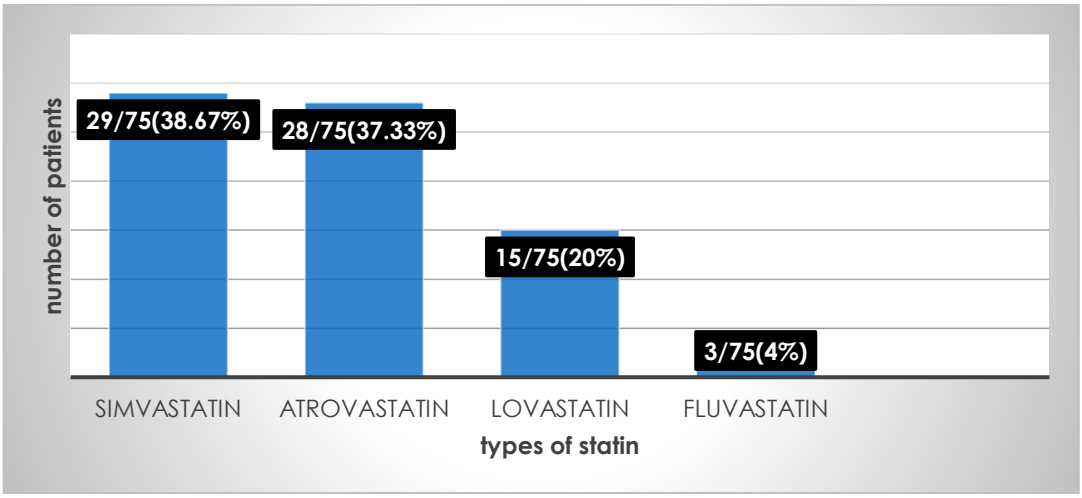


Figure 5: Frequency of statin type prescription and used by study participants at Diabetic Clinic TASH, Addis Ababa, 2017.

This study indicated that metformin was the most frequently prescribed 44/100 (44%) anti-glycemic drugs followed by insulin 19/100 (19%) among the study participants. And around 37/100 (37%) of the participants were on combination of anti-glycemic drugs (fig. 6)

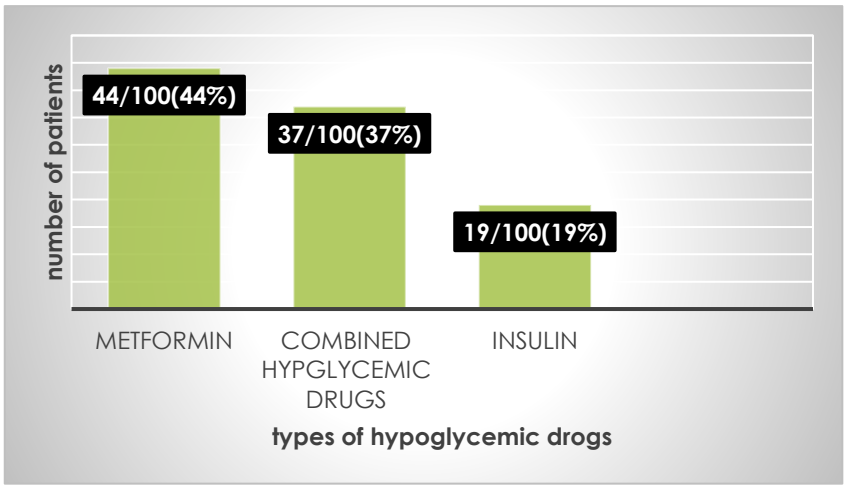


Figure 6: Frequency of hypoglycemic drugs among the study participants at Diabetic Clinic of TASH, Addis Ababa, 2017.

None of the study participants in this study was on highest dose (80mg) statin therapy. About 40/75 (53.3%) and 35/75 (46.7%) of the study participants in this study used 20mg and 40mg statin drugs respectively (fig. 8)

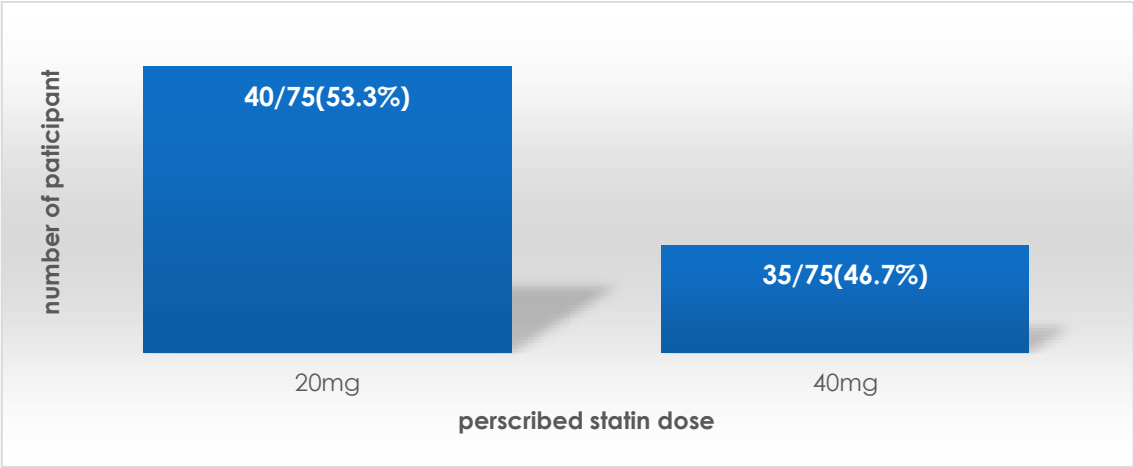


Figure 7: Frequency distribution of statin dose among study participants at Diabetic Clinic TASH, Addis Ababa, 2017.

Mean SBP, DBP, BMI and WHR were not significantly different among the four study groups. One way ANOVA indicated that mean SBP in T2DM patients who were on statin drugs for >18 months was higher (142mmHg) as compared with other study groups. Groups of study participants who were on statin for 14 days- 6 months and who were not on statin therapy had similar mean SBP. Mean DBP in all study groups was found in normal range. Mean values of BMI and WHR were higher than normal reference range in all study groups (table 1).

Table 2: Anthropometric and clinical characteristics of T2DM patients who were on statin drugs for 14 days-6 months, 6 months-18 months, greater than 18months and didn't on statin therapy at Diabetic Clinic of TASH, Addis Ababa, 2017.

Variable	Groups	Mean ± SD	P-value
SBP	14 days - 6 months(n=25)	136.40±14.40	0.418
	6 months-18months(n=25)	133.44±15.19	
	>18months(n=25)	142.00±20.62	
	not on statin therapy(n=25)	136.80±21.55	
	Total(N=100)	137.16±18.20	
DBP	14 days - 6 months(n=25)	80.40±6.76	0.494
	6 months- 18months(n=25)	79.36±9.59	
	>18months(n=25)	82.76±9.33	
	not on statin therapy(n=25)	82.80±12.08	
	Total(N=100)	81.33±9.60	
BMI	14 days - 6 months(n=25)	26.43±3.10	0.052
	6 months-18months(n=25)	26.69±3.73	
	>18months(n=25)	28.99±5.89	
	not on statin therapy(n=25)	26.00±2.99	
	Total(N=100)	27.03±4.20	
WHR	14 days - 6 months(n=25)	.96±.10	0.754
	6 months-18months(n=25)	.95±.06	
	>18months(n=25)	.97±.10	
	not on statin therapy(n=25)	.97±.06	
	Total(N=100)	.96±.07	

SBP= Systolic blood pressure, DBP= Diastolic blood pressure, BMI = Body mass index, and WHR= Waist-hip-ratio. Significance was considered at $p < 0.05$.

Mean values of LDL, TAG and total cholesterol had a statistically significant difference among the four study groups as determined by one way ANOVA**.** Post hoc Tukey test revealed that total cholesterol and TAG were significantly lower in group III than group I (P=0.019 &0.01), LDL was significantly lower in group III than group I (P=0.022) and group IV (P=0.027). But HDL- cholesterol and fasting blood glucose didn't have statistically significant difference among the study groups. Serum mean value of HDL-Cholesterol was lower than normal value while blood glucose was higher than normal value for all study groups (table 3).

Table 3: Characteristic features of lipid profile and blood glucose level in T2DM patients who were on statin therapy for 14days-6months, 6-18months, >18months and didn't on statin therapy at Diabetic Clinics of TASH, Addis Ababa, 2017.

Variables	Groups	Mean ± SD	P-value
TC	14 days - 6 months (n=25)	216.04±59.36 **G=III(P=0.019))**	0.022
	6 months – 18months(n=25)	186.16±50.89	
	>18months(n=25)	172.48±46.57**(G I(P=0.019))**	
	not on statin therapy (n=25)	202.56±49.55	
	Total(N=100)	194.31±53.62	
HDL-C	14 days - 6 months(n=25)	34.60±9.60	0.820
	6 months-18months(n=25)	32.56±10.36	
	>18months(n=25)	35.28±13.749	
	not on statin therapy (n=25)	34.64±8.12	
	Total(n=100)	34.27±10.547	
LDL-C	14 days - 6 months(n=25)	139.88±41.75 **(G III(P=0.022))**	0.008
	6 months-18months(n=25)	118.48±38.90	
	>18months(n=25)	107.60±35.364**(G I &IV(P=0.022 &0.027))**	
	not on statin therapy (n=25)	139.16±40.21**(G III(P=0.027)**	
	Total(N=100)	126.28±40.94	
TAG	14 days - 6 months(n=25)	233.84±108.146**(G III(P=0.01))**	0.018
	6 months-18months(n=25)	191.64±89.75	
	>18months(n=25)	148.24±69.87**(G I(P=0.01))**	
	not on statin therapy(n=25)	177.56±107.29	
	Total(N=100)	187.82±98.61	
FBG	14 days - 6 months(n=25)	167.24±53.44	0.662
	6 months-18months(n=25)	164.80±48.87	
	>18months(n=25)	169.60±76.79	
	not on statin therapy (n=25)	185.60±73.98	
	Total(N=100)	171.81±63.99	

Key: TAG= Triacylglycerol, LDL= Low density lipoprotein, HDL= High density lipoprotein, TC= Total cholesterol FBG=Fasting Blood glucose. Significance was considered at $p < 0.05$.

In this study mean level of serum liver enzymes (ALT, AST and ALP) were shown slight elevation among T2DM patients who were on statin therapy for greater than 18monthsas compared to other study groups but, statistically not significant. Means values of both serum ALT and AST in all study groups were found within normal reference range. But mean level ALP was higher than normal reference range in all study groups (table 4)

Table 4: Comparison of liver enzymes (ALT, AST and ALP) in T2DM patients who were on statin therapy for 14 days-6 months, 6 months-18 months, greater than 18 months and who were not on statin at TASH, 2017.

Variable	Groups	Mean ± SD	P-value
ALT	14 days - 6 months(n=25)	23.08±14.1	0.452
	6 months -18months (n=25)	19.88±6.8	
	>18months (n=25)	23.92±8.9	
	not on statin therapy (n=25)	21.2±10.4	
	Total(N=100)	22.2±10.3	
AST	14 days - 6 months(n=25)	20.2±7.9	0.638
	6 months -18 months (n=25)	19.6±4.9	
	> 18 months (n=25)	22.6±6.9	
	not start statin(n=25)	19.4±10.7	
	Total(N=100)	20.3±7.9	
ALP	14 days - 6 months (n=25)	195.7±45.4	0.189
	6 months -18 months (n=25)	187.2±57.2	
	> 18 months (n= 25)	215.7±74.3	
	not start statin(n=25)	184.6±36	
	Total (N=100)	195.6±55.7	

ALT= Alanine aminotransferase, AST= Aspartate aminotransferase, ALP= Alkaline phosphatase, n= Numbers participants in each groups and N= Total study subjects. Significance was considered at $p < 0.05$.

As one way ANOVA showed in this study serum level of CK-MB isotype was higher among T2DM patients who were on statin therapy for greater than 18months as compared with other study groups but not statistically significant. For all study groups serum mean value of CK-MB isotype was found with in normal reference range (CK-MB< 25 U/L). Mean value of LDH was higher among T2DM patients who were on statin for 14days-6months and greater than 18months but, statistically not significant. For all study groups mean values of LDH was found with in normal reference range but it was pulled to the upper limit (table 5).

Table 5: Comparison of LDH and CK-MB isotype among T2DM patients who were on statin drugs for 14 days-6 months, 6 months-18 months, greater than 18 months and who were not on statin therapy at Diabetic Clinic of TASH, Addis Ababa, 2017.

Variable	Groups	Mean ±SD	*P-value*
LDH	14 days - 6 months (n=25)	**191.6±77.9**	0.270
	6 months -18months (n=25)	174.3±38.7	
	>18months(n=25)	**191.7±32.3**	
	not start statin(n=25)	168.8±44.1	
	Total (N= 100)	181.6±55.7	
CK-MB	14 days - 6 months(n=25)	13.6±13.0	0.652
	6 months -18months(n=25)	15.3±5.3	
	>18months(n=25)	**17.01±8.0**	
	not start statin(n=25)	14.7±10	
	Total (N=100)	15.2±9.7	

***Key*:** LDH = Lactate dehydrogenase, CK-MB isotype = Creatine kinase of heart muscle isotype, n= Numbers of study participants in the study groups, N= Total numbers of study participants.

As table 6 indicates, mean values of BMI, WHR, DBP and SBP were not significantly different among T2DM patients who were on statin as compared to T2DM patients who were not on statin therapy. Mean values of SBP and DBP were slightly higher for female T2DM who were not on statin therapy in relation to female T2DM patients who were on statin drugs (*p = 0.153 & 0.116*) and mean value of WHR for both male and female T2DM who were on statin and who were not on statin drugs was higher than normal value (table 6).

Table 6: Comparison of anthropometric and clinical features of T2DM patients who were on statin therapy and T2DM patients who were not on statin therapy at Diabetic Clinics of TASH, 2017.

Variable	Male T2DM (n=48)			Female T2DM patients (n=52)		
	On statin (n=36)	Not on statin (n=12)	*p-value*	On statin (n=39)	Not on statin (n=13)	*p-value*
BMI mean ±SD	25±3.7	24.5±2.5	0.399	29±4.5	27.4±2.8	0.200
WHR mean ±SD	0.97±0.06	0.99±0.069	0.265	0.954±0.08	0.955±0.08	0.958
SBP mean ±SD	135±15.5	125.8±17.8	0.055	138±18.6	146.9±20	0.153
DBP mean ±SD	80.6±7.9	79.2±12.4	0.65	81±9.2	86±11.2	0.116

Key: BMI= Body mass index, DBP= Diastolic blood pressure, SBP= Systolic blood pressure, WHR= Waist-to-hip ratio and SD = Standard deviation. Significance was considered at $p < 0.05$.

As indicated in the table 7, liver enzymes (ALT, AST and ALP), CK-MB and LDH showed moderate elevation among T2DM patients who were on statin therapy as compared to T2DM patients who were not on statin therapy in both sex. For both male and female T2DM patients, TC, LDL cholesterol and TAG were lower among statin user as compared to non-statin user. With respect to lipid profile, female T2DM patients produced better response to statin than male T2DM patients.

Table 7: comparison of liver enzymes, CK-MB, LDH and lipid profile among male and female T2DM patients who were on statin therapy with T2DM patients who were not on statin therapy at diabetic clinic of TASH, Addis Ababa, 2017.

Variable mean ± SD	Male T2DM (n=48)			Female T2DM patients(n=52)		
	On statin (n=36)	Not on statin (n=12)	p-value	On statin (n=39)	Not on statin (n=13)	p-value
ALT	24±10.5	20.7±9.7	0.797	21.1±10.3	20.01±11.4	.862
AST	22±7	20±8.5	0.339	22.3±6.3	19±12.7	0.908
ALP	192±41.5	178±28	0.42	206±73.8	189±43	0.459
CK-MB	16.7±11.6	15.8±12	0.900	14.5±6.2	13.7±8.9	0.761
LDH	194±66.8	172±50.4	0.297	178.1±36.6	165.8±39	0.304
TC	189.25±41.6	204.39±50.7	0.356	179.72±56.7	214.85±54.6	0.057
LDL	125.42±29.6	131.19±38.2	0.635	113.49±41.3	151.85±45.4	0.007*
HDL	35.75±11.1	35.17±8.3	0.869	32.67±11.3	34.15±8.2	0.666
TAG	152.17±77.4	204.44±90.6	0.08	179.05±100.4	201.00±127.6	0.527

ALP= Alkaline phosphatase, ALT = Alanine aminotransferase, AST= Aspartate aminotransferase, BD =Blood glucose, CK-MB = Creatine kinase heart isotype, HDL-C=High density cholesterol, LDH =Lactate dehydrogenase, LDL-C= Low density lipoprotein cholesterol, SD= Standard deviation TAG= triacylglycerol, TC= Total cholesterol, * =statistically significant difference. Significance was considered at $p < 0.05$.

There was an elevation of serum total cholesterol and LDL among T2DM patients who were not on statin therapy as compared T2DM patients who were on statin therapy. Mean values of liver enzymes (ALT, AST and ALP), CK-MB isotype and LDH were slightly higher among T2DM patients who were on statin therapy than T2DM patients who were not on statin therapy but, statistically not significant. HDL-cholesterol was more or less similar for the two study groups (table 8).

Table 8: Comparison of biochemical parameter among T2DM who were on statin and T2DM who were not on statin therapy at Diabetic Clinic of ATSH, Addis Ababa, 2017.

Parameter	T2DM who were on statin (n=75)	T2DM who were not on statin (n=25)	***p-value***
TC mean ±SD	192±54.9	203±49.5	0.377
LDL-C mean ±SD	122±40.5	139±40.2	0.069
HDL-C mean ±SD	34.15±11.3	34.64±8.2	0.841
TAG mean ±SD	177±96	191±107	0.551
FBG mean ±SD	167±60	185±73.9	0.215
ALT mean ±SD	22.49±10.4	21.2±10.6	0.593
AST mean ±SD	20.6±6.7	19.5±10.7	0.540
ALP mean ±SD	199.4±60.5	184.2±36.4	0.237
CK-MB mean ±SD	15.3±9.4	13.7±10.9	0.587
LDH mean ±SD	185.88±53.5	168.7±44.1	0.152

ALP= Alkaline phosphatase, ALT = Alanine aminotransferase, AST= Aspartate aminotransferase, BD =Blood glucose, CK-MB = Creatine kinase heart isotype, HDL-C=High density cholesterol, LDH =Lactate dehydrogenase, LDL-C= Low density lipoprotein cholesterol, SD= Standard deviation TAG= triacylglycerol, TC= Total cholesterol. Significance was considered at $p < 0.05$.

Mean values of total cholesterol, LDL and TAG were higher among T2DM patients who were on 20mg statin therapy as compared to T2DM patients who were on 40mg statin therapy but, statistically not significant. Mean values of liver enzymes (ALT, AST and ALP), CK-BM isotype and LDH were slightly lower among T2DM patients who were on 20mg statin therapy than T2DM patients who were on 40mg statin therapy but, statistically they were not significant(table 9).

Table 9: Comparison of biochemical parameter among T2DM patients who were on 20mg statin therapy and T2DM patients who were on 40mg statin therapy at Diabetic Clinics of TASH, Addis Ababa, 2017.

Parameter	T2DM who were on 20mg statin (n=40)	T2DM who were on 40mg statin (n=35)	*p-value*
TC mean ±SD	203.6±57	181±51	0.076
LDL-C mean ±SD	131±41	114±38	0.071
HDL-C mean ±SD	33.4±11.6	35±11	0.530
TAG mean ±SD	209.6±112	175±76	0.122
FBG mean ±SD	174±46	160.5±70	0.308
ALT mean ±SD	20.68±11.0	24.57±9.4	0.107
AST mean ±SD	20.0±7.3	20.94±5.9	0.682
ALP mean ±SD	196.7±37.5	202.2±75.5	0.670
CK-MB mean ±SD	14.4±6.3	15.7±11.4	0.654
LDH mean ±SD	175.2±34	195.2±64	0.106

ALP= Alkaline phosphatase, ALT = Alanine aminotransferase, AST= Aspartate aminotransferase, BD =Blood glucose, CK-MB = Creatine kinase heart isotype, HDL-C=High density cholesterol, LDH =Lactate dehydrogenase, LDL-C= Low density lipoprotein cholesterol, SD= Standard deviation TAG= Triacylglycerol, TC= total cholesterol. Significance was considered at $p < 0.05$.

One way ANOVA in this study indicated that mean values of serum total cholesterol and LDL cholesterol had statistically significant difference among T2DM patients who were on metformin + statin, T2DM patients who were on insulin + statin therapy and T2DM patients who were on combined hypoglycaemic drugs + statin. (*P-value=0.034* for total cholesterol and *0.046* for LDL-cholesterol). A Tukey Post hoc test indicates that mean values of serum total cholesterol and LDL cholesterol were significantly higher among T2DM patients who were on metformin + statin therapy than T2DM patients who were on insulin + statin therapy. Mean value HDL cholesterol was higher in T2DM patients who were on insulin + statin therapy than to other two groups. Mean values of serum glucose, liver enzymes (ALT, AST and ALP), CK-MB isotype and LDH were not significantly different among the three groups (table 10).

Table 10: comparison of lipid profile, blood glucose, liver enzymes, CK-MB and LDH among T2DM patients who were on metformin + statin, insulin + statin and combined hypoglycemic drugs + statin therapy at diabetic clinic of TASH, Addis Ababa, 2017

Parameter	Treatment			***p-value***
	Metformin + statin (n=34)	Insulin + statin (n=17)	Combined hypo-glycemic + statin (n=24)	
TC mean±SD	209.±54.5	171.6±50	191±54.9	0.034*
LDL-C mean ±SD	138±40	110±36	112±39.9	0.046*
HDL-C mean ±SD	29.9±12.8	36±10.8	33.2±10	0.092
TAG mean ±SD	205.9±100	153.2±51	197.4±109	0.169
FBG mean ±SD	174±63.4	147±67	170.6±52	0.936
ALT mean ±SD	22.18±10	19.94±8	24.75±12	0.342
AST mean ±SD	21.5±7.3	18.71±4.3	20.3±7.1	0.372
ALP mean ±SD	208.3±67	203.2±75	184.2±31	0.317
CK-MB mean ±SD	16.7±12	14.2±7.3	14.3±5.9	0.508
LDH mean ±SD	193±69.4	190.88±34.2	171±33.6	0.254

Key: ALP= Alkaline phosphatase, ALT = Alanine aminotransferase, AST= Aspartate aminotransferase, BD =Blood glucose, CK-MB = Creatine kinase heart isotype, HDL-C=High density cholesterol, LDH =Lactate dehydrogenase, LDL-C= Low density lipoprotein cholesterol, SD= Standard deviation, TAG= Triacylglycerol, TC= Total cholesterol, * statistically significant difference. Significance was considered at $p < 0.05$.

CHAPTER FIVE

Discussion

Diabetes is the most common metabolic disorder worldwide and its prevalence has become higher in developing countries; it is common in Ethiopia (World Health Organization, 2016). T2DM initially involving insulin resistance then leads to hyperinsulinemia and eventually progresses to involve pancreatic beta cell dysfunction that leads to insulin deficit is the most prevalent form of diabetes. More than 90% of all newly diagnosed diabetic cases are T2DM (Kahn *et al*., 2014). Dyslipidemia is one of the most characterized metabolic syndrome as a result of diabetes, especially T2DM. This makes T2DM patients considered as high risk groups for developing CVD (Kawanami *et al*., 2016, Wu & Parhofer, 2014). As a result, all T2DM above age of 40 years are recommended to take anti-dyslipidemic drugs, mainly statin (Balgi *et al.*, 2017), and the minimum age of the participant in this study was 38 years old (table 1).

HMG-CoA reductase inhibitors, more commonly known as statins, are the most widely used medications for decreasing LDL cholesterol and play a vital role in the prevention of atherosclerotic cardiovascular complications. Available statins for prescription are: simvastatin, atorvastatin, fluvastatin, pravastatin, rosuvastatin and lovastatin (Pedro-Botet *et al.*, 2016).

In the present study, simvastatin, atorvastatin and lovastatin were prescribed for 29(38.7%), 28(37.3%) and 15(20%) of total 75 study participants respectively. Statin works by inhibiting HMG-CoA reductase, causing upregulation of the LDL-C receptors on the surface of the liver cell and increasing the removal of LDL-C from the blood. HMG-CoA reductase is rate limiting enzymes in cholesterol biosynthesis. In addition, statins lower serum triglycerides concentration and modify endothelial function, inflammatory responses, plaque stability, and thrombus formation. As a result, statin appears to play a major role in decreasing the risk of coronary heart disease (CHD) and all-cause of mortality (Trentman *et al.*, 2016, Bonetti *et al.*, 2003).

The results of the present study indicated that the mean serum values of total cholesterol, LDL-cholesterol and TAG were significantly different among the four study groups (group I, II, III and IV). Post hoc Tukey's multiple comparison test revealed that total cholesterol and TAG concentration were significantly lowered in group III as compared to group I (p=0.019, 0.01). In addition, LDL cholesterol was significantly lowered in group III than in group I (P=0.022)

and group IV (P=0.027) (table, 3). Similarly, mean serum level of LDL-cholesterol was lowered in T2DM patients who were on statin therapy than in T2DM patients who were not on statin therapy (P=0.069) (table 8). This may be due to the fact that statin inhibits cholesterol biosynthesis in the liver and promotes LDL-cholesterol clearance (Trentman *et al.*, 2016; Stancu and Sima, 2001). Two-third of cholesterol in biological system comes from endogenous sources and the rest 1/3 comes from the food we eat (exogenous source). In normal condition, regulation of body cholesterol level targets the endogenous sources. Control of cholesterol biosynthesis could be achieved by regulation of HMG-CoA reductase gene transcription (feedback repression), hormonal regulation (insulin and glucagon) that mediate enzyme dephosphorylation and phosphorylation and competitive inhibition of HMG- CoA reductase by statin. Liver is a site for cholesterol and TAG synthesis, VLDL and HDL formation and assembly plus statin action and all these reasons may be responsible for the result (Fitzakerley, 2005). The result of this study was in line with studies done by Trentman *et al. (*2016) and Stancu and Sima, (2001) to assess the efficacy of statin drugs. Other studies done to investigate the long term efficacy and safety of statin therapy by Takata *et al*, (2017), Ford *et al*., (2016) and Lu *et al*., (2016) support this result. The mean value of all serum lipid panel in all study groups were higher than normal reference range, which may be due to T2DM. This is supported by study done by Borle *et al.* (2016) to assess dyslipidemia in T2DM patients and that showed (86%) had dyslipidemia. Independent t-test in this study indicated that mean values of serum total cholesterol, LDL cholesterol and TAG were higher among T2DM patients who were on 20mg statin as compared to T2DM patients who were on 40mg statin therapy (P-values= 0.076 for total cholesterol, 0.071 for LDL cholesterol and 0.122 for TAG) (table 8). This result agrees with study done by Karlson *et al.* (2017) and Pedro-Botet *et al*. (2016) that indicated high dose statin is responsible to achieve desired plasma lipid goal. As the statin dose increase the potency to decrease the total cholesterol, LDL cholesterol and TAG increase linearly. The mean values of serum total cholesterol and LDL cholesterol in this study were significantly different among T2DM patients who were on metformin + statin, insulin + statin and combined hypoglycemic drugs + statin therapy (P=0.034 for total cholesterol and P=0.046 for LDL) (table 10). These may be due to better glucose control potential and multiple effect of insulin in the metabolism of glucose and fat by muscle and other tissue (Rotella *et al*., 2013). Duration of statin usage among metformin user and the smaller the sample size may also be another factor.

The anatomic and physiologic nature of liver makes it central to metabolism of virtually every foreign substance including most drugs like statin (Han *et al.*, 2017; Kullak-Ublick *et al*., 2017,

Marrone *et al*., 2017). Results of the present study showed that the mean values of serum liver enzymes (ALT, AST and ALP) had shown statistically non-significant elevation among T2DM patients who were on statin for greater than 18months as compared to other groups. Both mean serum ALT and AST in this study were found to be within the normal reference range in all of study groups (table 3). However, this study result was not strongly agree with study done by Dragos *et al***.** (2017) that revealed much increased levels of aminotransferases in patients who were on atorvastatin therapy for 8 months. Clinical trials done by Thapar *et al.* (2013) have shown that statin therapy has been associated with elevations in serum alanine aminotransferase (ALT) levels in approximately 3% of persons who have received statin drugs. Study done by Gao *et al.* (2017) in China also indicated that ALT >1 × ULN was higher among statin users. Absence of significant liver enzymes (ALT, AST and ALP) elevation among statin user at different time periods in this study may be due to well tolerability of statin by the study participants and none of the study participants used highest statin dose (53.3% and 46.7% of the study participant used 20mg and 40mg statin respectively) (figure 9). In addition, none of the participants in this study was chronic alcoholic, cigarette smoker and took drug other than hypoglycemic and statin drugs. All the above factors have impact on liver function and exacerbate adverse effect of statin. The mean serum value of ALP was above normal reference range in all the study groups (table 3). Elevation of mean serum ALP in all of this study groups may be due dyslipidemia as all groups of study participants had higher mean lipid profile and poor blood glucose control (table 1& 2).

Liver enzymes (ALT, AST and ALP) shown statistically non-significant elevation among T2DM patients who were on statin therapy as compared to T2DM patients who were not on statin therapy (table 8). These elevations of liver enzymes could be due to all statin target liver for its antidyslipidemic effect and metabolism and its interaction with hypoglycemic drugs; thereby inducing mild hepatotoxicity (Björnsson, 2016; Clarke et al., 2016; Stancu and Sima, 2001). Similarly, serum mean values of liver enzymes (ALT, AST and ALP) were higher among T2DM patients who were on 40mg statin therapy than T2DM patients who were on 20mg statin therapy (table 9). These may be due to dose dependent idiosyncratic drug-induced liver injury and its pathogenesis is complex that depends on different factors (Ortega-Alonso *et al.*, 2016). This result is in line with study done by Dragos *et al.* (2017) to evaluate level of liver enzymes of patients who were on highest dose atorvastatin therapy for 8 months that revealed much increased levels of aminotransferases. Mean values of liver enzymes (ALT, AST and ALP) among T2DM patients who were on metformin + statin, insulin + statin and combined

hypoglycemic drugs + statin were not significantly different (table 10). This may be due to the smaller the sample size, study design effect and well tolerability of hypoglycemic drugs and statin and their interaction by the liver.

Quantitative measurement of serum CK-MB isotype has been reported to be useful for the diagnosis of myocardial infarction, re-infarction, and the size of infarction. CK-MB can also be used as a secondary marker to aid in the diagnosis and measuring the degree of myocardial necrosis as a result of drug toxicity (Egholm and Pareek, 2015). Of the total CK found in the serum, 6-25 % is CK-MB isotype. The finding of this study indicated that the mean serum level of CK-MB isotype was non- significantly higher among T2DM patients who were on statin for greater than 18 months as compared to other study groups, and the result was within normal reference range (table 4). This result is supported by study done by Parker *et al***.** (2013) to measure serum CK before and after atorvastatin or placebo was administered for 6 months to 420 healthy, statin-naive subjects and indicated no individual CK value ≥10× ULN. Other study done by Soko *et al*. (2016) in Zimbabwean didn't show any elevation of serum CK. However, reviews done by Stroes *et al. (*2015) showed there was statin-associated myopathy with significant elevation of serum CK. Other study done by Calza *et al*. (2017) showed that statin induced elevation of CK is positively related with duration of statin use. The mechanism by which statin induces muscle cell injuries is by impaired synthesis of coenzyme Q10 (ubiquinone) for mitochondrial function, depletion of isoprenoids and disruption of cell membrane integrity (Ambapkar *et al.,* 2016). These variations between those studies could be genetic and environmental factors. In addition, none of study participant in this study use high dose (80mg) of statin drugs, chronic alcohol drinker, cigarette smoker and took drugs other than hypoglycemic and statin. The above factors can exacerbate the myotoxicity especially cardiotoxicity of statin which is characterized by increased serum level of CK-MB isotype.

Mean value of serum CK-MB isotype level among T2DM patients who were on statin therapy showed non-significant elevation as compared to T2DM patients who were not on statin therapy (table 8). The cause for serum CK elevation could be quite diverse, including excessive muscular stress and ischemia, genetic defects, and direct toxic or physical damage, drugs and alcohol (Holder, 2016). Statin drugs can disrupt the normal cardiac myocyte membrane integrity leading to release of intracellular components including structural proteins and enzymes such as CK-MB isotype in serum (Egholm and Pareek, 2015). Similarly, CK-MB isotype among T2DM patients who were on 40mg statin was higher as compared to T2DM patients who were on 20mg statin therapy (table 9). This non-significant elevation of CK-MB

isotype among T2DM patients who were on 40mg statin could be dose dependent statin myotoxicity. Study done by Auer *et al*. (2016) showed as the doses of statins administered for patients increase, muscle-related side-effects will become more prevalent. Serum mean value of CK-MB isotype among T2DM patients who were on metformin +statin, insulin + statin and combined hypoglycemic drugs were not significantly different (table 10). This may be due the smaller the sample size of this study and/or the safe interaction of statin with hypoglycemic drugs.

Cellular enzymes in the extracellular space serve as indicators of disturbances of the cellular integrity induced by pathological conditions. Lactate dehydrogenase (LDH) is a cytoplasmic enzyme present in essentially all major organ systems. Ingestion of certain drugs, toxin and chemical poisons are among the major factors for LDH release to extracellular space (Mohammed & Elias, 2016). Elevated serum LDH could act as a biomarker for diagnosis of drug induced hepatic and muscular injury. As previously published works done by Noor *et al*. (2016) and Tokinaga *et al.* (2006) showed that statin therapy is responsible for moderate to severe elevation of serum LDH. The result of this study revealed that the mean serum level of LDH among T2DM patients who were on statin therapy for 14 days-6 months and >18 months showed non-significant elevation as compared to T2DM patients who were on statin for 6-18months and not on statin therapy. And the result was within normal reference range (table 4). Previously published works done by Noor *et al. (*2016) and Tokinaga *et al.* (2006) reported that statin drugs can increase serum LDH through disruption of cellular integrity, especially when it has been taken together with other drugs. Independent t-test in this study showed there was moderate elevation of mean serum LDH level among T2DM patients who were on statin therapy than T2DM patients who were not on statin therapy (table 8). In addition, mean serum values of LDH among T2DM patients who were on 40mg statin therapy was higher than T2DM patients who were on 20mg statin therapy (table 9). This result was supported by case report done by Dragos *et al.* (2017) that showed elevation of LDH in T2DM patients who were on statin therapy. Other case report done by Unnikrishnan and Satish, (2005) indicated that taking atorvastatin for several years elevate serum CK and LDH.

CHAPTER SIX

Conclusion

Statin drugs were found to have the effect of lowering lipid profiles (total cholesterol, LDL-cholesterol and TAG) in T2DM patients who were on statin therapy. The effects were found to be prominent in T2DM patients who were on statin drugs for greater than 18 months. In addition, 40mg statin dose produced better effect on lipid profile as compared to 20mg statin dose in T2DM patients. In this study insulin + statin shows better results on the nature of lipid profiles.

The mean serum level of liver enzymes (ALT, AST and ALP), CK-MB isotype and LDH didn't show significant difference among T2DM patients who were on statin for different time periods. Moderate elevation of liver enzymes, CK-MB and LDH were observed among T2DM patients who were on statin therapy as compared to T2DM patients who were not on statin therapy. The mean values of ALT and AST were found within normal reference range in all study groups. ALP was higher than normal values among all study groups.

CHAPTER SEVEN

STERENGTH, LIMITATIONS AND RECOMMENDATION

7.1. Strength of the present study

- Socio-demographic data and sample collection was done by well experienced nurses with strict following of standard procedure.
- Biochemical parameter analysis were done by fully automated instrument.
- Data entry and statistical analysis were done by recent software SPSS 22.

7.2. The limitations in the present study include

- Small study sample size, lack of measuring more biochemical parameters to assess full liver function and muscle (kidney and cardiac) condition of T2DM patients who were on statin.
- Also, this short cross- sectional study could not follow up the patients, who were taking statin drugs for long duration time for biochemical and enzymatic progression due to limitation of money and time.

7.3. The following recommendations are forwarded

- Health institutions and researcher should plan further study with large sample size to investigate effects of statin drugs on liver functions and muscle condition among T2DM
- Similar studies with wider parameter must be done on different cases like HIV patients, cardiac patients …
- To understand short and long term effect of statin therapy prospective and cohort studies should be in place

REFERENCES

Abebe SM, Berhane Y, Worku A, Alemu S (2013) Increasing Trends of Diabetes Mellitus and Body Weight: A Ten Year Observation at Gondar University Teaching Referral Hospital, Northwest Ethiopia. *PLoS ONE* 8(3): e60081.

Alberti K., Zimmet P. and Shaw J. (2007). International Diabetes Federation: a consensus on Type 2 diabetes prevention. *Diabet. Med.* 24: 451–463.

Alemu F. (2015). Prevalence of Diabetes Mellitus Disease and its Association with Level of Education Among Adult Patients Attending at Dilla Referral Hospital, Ethiopia. *J Diabetes Metab*. 6(4): 521.

Ambapkar S. N Shetty N. & Dwivedy A., (2016). Statin-Induced Rhabdomyolysis. *J Associ Physi India.* 64: 93-4.

American Diabetes Association, (2016). Erratum. Classification and diagnosis of diabetes. Sec. 2. In Standards of Medical Care in Diabetes–2016. *Diabetes Care.* 39 (1): S13–S22.

American Diabetes Association, (2017). Erratum Classification and Diagnosis of Diabetes. Sec-2. *Diabetes Care*. 40(1):S11–S24.

Arca M. and Pigna G., (2011). Treating statin-intolerant patients. *Diabetes Metab Syndr Obes*, 4, pp.155-166.

Auer J., Sinzinger H., Franklin B. and Berent R. (2016). Muscle- and skeletal-related side-effects of statins: tip of the iceberg? *European Journal of Preventive Cardiologyl.* 23(1), 88–110.

Azhar S. (2010). Peroxisome proliferator-activated receptors, metabolic syndrome and cardiovascular disease. *Futur Cardiol*. 6(5): 657-691.

Bali K. & Vij A. S., (2016). Pattern of dyslipidemia in Type 2 Diabetes Mellitus in Punjab. *Int J Res Med Sci*. 4(3): 809-812.

Balgi V., Harshavardan L, Sahna E. & Thomas S. K. (2017). Pattern of Lipid Profile Abnormality in Subjects with Prediabetes. *International Journal of Scientific Study.* 4(11): 150-153.

Basak R.C., Chatterjee M. and Sarma P. (2013). An overview on management of diabetic dyslipidemia. *J Diabetes Endocrinol*. 4(3), 27-36.

Bitztur R., Cohen H., Kamari Y. and Harats D. (2013). Intolerance to Statins: Mechanisms and Management. *Diabetes care.* 36(2): S325-S330.

Björnsson E.S. (2016). Hepatotoxicity by Drugs: The Most Common Implicated Agents. *Int. J. Mol. Sci.* 17(2): 224.

Björnsson E, Jacobsen E.I. & Kalaitzakis E. (2012). Hepatotoxicity associated with statins: Reports of idiosyncratic liver injury post-marketing. *J Hepatol.* 56(2):374–380.

Bonetti P.O., Lerman L.O., Napoli C. and Lerman A., (2003). Statin effects beyond lipid lowering—are they clinically relevant? European heart journal, 24(3): 225-248.

Borle A. L., Chhari N., Gupta G., Bathma V. (2016). Study of prevalence and pattern of dyslipidaemia in type 2 diabetes mellitus patients attending rural health training centre

of medical college in Bhopal, Madhya Pradesh, India. *Int J Community Med Public Health*. 3(1):140-144.

Calza L., Magistrelli E., Colangeli V., Borderi M., Contadini I., Bon I., Carla Re M. and Viale P. (2017). Significant association between statin-associated myalgia and vitamin D deficiency among treated HIV-infected patients. AIDS. 31:681–688.

Catapano A.L., Graham I., De Backer G., Wiklund O., Chapman M.J., Drexel H., Hoes A.W., Jennings C.S., Landmesser U., Pedersen T.R. and Reiner Ž., (2016). 2016 ESC/EAS Guidelines for the Management of Dyslipidaemias. *European heart journal*, 37(39): 2999-3058.

Cefalu W. T. (2017). Standards of medical care in diabetes—2017. Diabetes Care abridged for primery care provider. *Diabetes care*. 40(1): S1-S132.

Centers for Disease Control and Prevention, 2017. National diabetes statistics report: estimates of diabetes and its burden in the United States, 2017. Atlanta, GA: US Department of Health and Human Services, 2017.

Chehade J.M., Gladysz M. and Mooradian A.D., (2013). Dyslipidemia in type 2 diabetes: prevalence, pathophysiology, and management. *Drugs*. 73(4): 327-339.

Choudhary M. and Vyas R.K., (2015). A study of liver dysfunction determinants in normal and type 2 diabetic subjects in indian population. *Int J Sci Res.* 4(2): 2277-8179.

Clarke A.T., Johnson P.C., Hall G.C., Ford I. and Mills P.R., (2016). High dose atorvastatin associated with increased risk of significant hepatotoxicity in comparison to simvastatin in UK GPRD cohort. *PloS one.* 11(3): e0151587.

Coco T.J. and Klasner A. E. (2004). Drug-induced rhabdomyolysis. *Curr Opin Pediatr*. 16: 206–210.

Collins R., Reith C., Emberson J., Armitage J., Baigent C., Blackwell L., Blumenthal R., Danesh J., Smith G.D., DeMets D. and Evans S., (2016). Interpretation of the evidence for the efficacy and safety of statin therapy. *The Lancet*. 388(10059): 2532-2561.

de Vries F. M. (2016). Statin treatment in type 2 diabetes patients [Groningen]: Rijksuniversiteit Groningen.

Diaz-Valencia P.A., Bougnères P. and Valleron A.J., (2015). Global epidemiology of type 1 diabetes in young adults and adults: a systematic review. *BMC Public Health*, 15(1): 255.

Dragos D, Pruteanu D and Constantin R. (2017). High-Dose Statin Associated with Rhabdomyolysis, Acute Kidney Injury, Cholestatic Liver Injury, and Thrombocytopenia. *J Nephrol Kidney Dis*. 1(1): 1001

Egholm G.and Pareek M. (2015). Drug-Induced Rhabdomyolysis with Elevated Cardiac Troponin T. *Case Reports in Medicine*. Article ID 270204, 3.

. Ekpe E. E. L and Omotoso A. J. (2015). The Relevance of Ascitic Lactate Dehydrogenase (LDH) and Serum Ascites Albumin Gradient (SAAG) in the Differential Diagnosis of Ascites among Patients in a Nigerian Hospital. *British Journal of Medicine & Medical Researc*h. 8(3): 211-219.

Elnaem M. H., Mohamed M. H., Huri H. Z., Azarisman S. M., & Elkalmi R. M. (2017). Statin Therapy Prescribing for Patients with Type 2 Diabetes Mellitus: A Review of Current Evidence and Challenges. *J Pharm Bio Sci.* 9(2): 80–87.

Faloppi L., Bianconi M., Memeo R., Casadei Gardini A., Giampieri R., Bittoni A., Andrikou K., Del Prete M., Cascinu S. and Scartozzi M., (2016). Lactate Dehydrogenase in Hepatocellular Carcinoma: Something Old, Something New. *BioMed Research International*, 2016.

Faloppi L., Del Prete M., Gardini A.C., Santini D., Silvestris N., Bianconi M., Giampieri R., Valgiusti M., Brunetti O., Bittoni A. and Andrikou K., (2016). The correlation between LDH serum levels and clinical outcome in advanced biliary tract cancer patients treated with first line chemotherapy. *Scientific reports*, 6.

Ferrara A. (2007). Increasing Prevalence of Gestational Diabetes Mellitus A public health perspective. *Diabetes Care*. 30(Supplement 2): S141-S146.

Fitzakerley J. (2005). Lipid lowering agents. *Cardiovascular system.* Med 307, 1-22.

Ford I., Murray H., McCowan C. and Packard C.J., (2016). Long term safety and efficacy of lowering LDL cholesterol with statin therapy: 20-Year Follow-Up of West of Scotland Coronary Prevention study. *Circulation*. Circulationaha-115.

Fowler M. J., (2008). Microvascular and Macrovascular Complications of Diabetes. *Clinical Diabetes.* 26 (2): 77-82.

Gao Y., Wang Y., Wu N.Q., Zhu C.G., Guo Y.L., Qing P., Liu G., Dong Q. and Li J.J., (2017). Statins use and the risk of liver dysfunction: A Chinese cohort study in real world clinical practice. *IJC Metabolic & Endocrine*. 16: 16–20.

Gencer B. & Mach F. (2016). Review of the 2016 European dyslipidaemia guidelines. *Cardiovascular medicine*. 19(11): 283–287.

George S., Patel M. and Stetz L., (2017): Diabetes: The world's weightiest problem: Volume IV

Guariguata, L., (2011). Estimating the worldwide burden of type 1 diabetes. *Diabetes Voice*, 56(2): 6-8.

Hall P., and J. (2012). Cash What is the Real Function of the Liver 'Function' Tests? *Ulster Med J*. 81(1): 30-36.

Han H., He Y., Hu J., Lau R., Lee H. and Ji C. (2017). Disrupted ER-to-Golgi Trafficking Underlies Anti-HIV Drugs and Alcohol-Induced Cellular Stress and Hepatic Injury. *Hepatology communications*. 1(2): 122-139.

Hennessy E., Adams C., Reen F.J. and O'Gara F., (2016). Statins as next generation anti-microbials: Is there potential for repurposing? *Antimicrobial Agents and Chemotherapy*, AAC-00192.

Holder K. (2016). Myalgias and Myopathies: Drug-Induced Myalgias and Myopathies. *FP Essent.* 440: 23-7.

Huang X-J., Choi Y-K., Im H-S., Yarimaga O., Yoon E. and Kim H-S. (2006). Aspartate Aminotransferase (AST/GOT) and Alanine Aminotransferase (ALT/GPT) Detection Techniques. *Sensors*, 6: 756-782

Huijgen H.J., Sanders G.T. B., Koster R. W., Vreeken J. and Bossuyt P. M. M. (1997). The Clinical Value of Lactate Dehydrogenase in Serum. *Eur J Clin Chem Clin Biochem*. 35(8): 569-579

Hussian A., Ali I., Ijaz M. and Rahim A. (2017). Correlation between HA1C and lipid profile in Afghani patients with type 2 diabetes. *Ther Adv Endocrinol Meta.* 8(4): 51-57

Imai K., Watari S., Sakazume T. and Mitsuyama S.(2008). Clinical Chemistry and Immunoassay Testing Supporting the Individual Healthy Life. *Hitachi Review*.57: 1-7

International Diabetes Federation, (2015). IDF diabetes atlas. Seventh edi.

International Diabetes Federation, (2014). Global Diabetes Scorecard Tracking Progress for Action. www.idf.org.

Jafari-Shobeiri M., Ghojazadeh M., Azami-Aghdash S., Naghavi-Behzad M., Piri R., Pourali-Akbar Y., Nasrollah-Zadeh R., Bayat-Khajeh P. and Mohammadi M., (2015). Prevalence and risk factors of gestational diabetes in Iran: a systematic review and meta-analysis. *Iranian journal of public health*, 44(8): 1036.

Jia G., DeMarco V.G. and Sowers J.R., (2016). Insulin resistance and hyperinsulinaemia in diabetic cardiomyopathy. *Nature Reviews Endocrinology*. 12(3): 144-153.

Jin Y. and She J.X. (2012). Novel biomarker in type 1 diabetes. *Rev Diabet Stud*, 9(4): 224-235.

Joo S.J., (2012). Anti-inflammatory effects of statins beyond cholesterol lowering. *Korean circulation journal,* 42(9): 592-594

Kahn S.E., Cooper M.E. and Del Prato S., (2014). Pathophysiology and treatment of type 2 diabetes: perspectives on the past, present, and future. *The Lancet*. 383(9922): 1068-1083.

Kalantari S. and Naghipour M., (2014). Statin therapy and hepatotoxicity: Appraisal of the safety profile of atorvastatin in hyperlipidemic patients. *Advanced biomedl res.* 3(1): 168.

Kansal S. & Kamble TK. (2016). Lipid Profile in Prediabetes. *Journal of The Association of Physicians of India.* 64: 18-21.

Karlson B.W., Palmer M.K., Nicholls S.J., Barter P.J. and Lundman P., (2017). Effects of age, gender and statin dose on lipid levels: Results from the VOYAGER meta-analysis database. *Atherosclerosis.* 265, 54-59.

Kawanami D., Matoba K.and Utsunomiya K. (2016). Dyslipidemia in diabetic nephropathy. *Renal Replacement Therapy*. 2:16

Keating G.M. and Croom K.F., (2007). Fenofibrate. *Drugs.* 67(1): 121-153.

Kiranmayi B., V. Bhavani and R. Tagore (2015). Evaluation of CK MB levels in Acute Ischemic stroke. *J Phar Bio* Sci. 10 (3): 38-42.

Krikorian A., (2016). Standards of medical care in diabetics. *J Ameri. Diabet associa*. 39 (Suppl. 1): S1-S2.

Kullak-Ublick G.A., Andrade R.J., Merz M., End P., Benesic A., Gerbes A.L. and Aithal G.P., (2017). Drug-induced liver injury: recent advances in diagnosis and risk assessment. *Gut, pp.gutjnl*-2016.

Kwiterovich P.O., (2003-2004). Laboratory Procedure Manual for Total Cholesterol, Direct HDL, Precipitated HDL, Triglycerides, and LDL. *Hitachi 704 Analyzer*.1-23

Lambert M., (2014). ACC/AHA Release Updated Guideline on the Treatment of Blood Cholesterol to Reduce ASCVD Risk. *American family physician*. 90(4): 260.

Leng J, Shao P, Zhang C, Tian H, Zhang F, Zhang S, et al. (2015) Prevalence of Gestational Diabetes Mellitus and Its Risk Factors in Chinese Pregnant Women: A Prospective Population-Based Study in Tianjin, China. *PLoS ONE* 10(3): e0121029 .

Lucaccioni L. & Iughetti L. (2016). Issues in Diagnosis and Treatment of Type 1 Diabetes Mellitus in Childhood. *Journal of Diabetes Mellitus.* 6: 175-183.

Lu Y., Cheng Z., Zhao Y., Chang X., Chen C., Bai Y. and Cheng N. (2016). Efficacy and safety of long term treatment with statin for coronary heart disease: A Bayesian network meta-analysis. *Atherosclerosis*. 254: 215-227.

Maji D., Shaikh S., Solanki D. and Gaurav K. (2013). Safety of statins. 17(4): 636-646

Marrone G., Vaccaro F.G., Biolato M., Miele L., Liguori A., Araneo C., Ponziani F.R., Mores N., Gasbarrini A. and Grieco A., (2017). Drug-induced liver injury: the diagnosis is not easy but always to keep in mind. *European review for medical and pharmacological sciences*, 21(1 Suppl): 122-134.

Mistire W.G., (2013). Diabetes mellitus and associated diseases from Ethiopian perspective: systematic review. *Ethiop. J. Health Dev*, 27(3): 249-253.

Model C.C., (2015). Standards of medical care in diabetes abridged for primary care providers. Diabetes care, 38(1): S1-S94.

Moghadam-kia S., Oddis C.V. and Aggarwal R. (2016). Approach to asymptomatic creatine kinase elevation. *Cleve Clin J Med*. 83(1): 37–42.

Mohammed N.B. & Elias S. E. (2016). Assessment of GammaGlutamyl Transferase and Lactate Dehydrogenase as Biochemical Markers of Preeclampsia Severity in Sudanese Pregnant Women'sin Khartoum State. *Savant J Med Sci.* 2(9): 177-183.

Mooradian AD., (2009). Dyslipidemia in type 2 diabetes mellitus. *Nat Clin Pract Endocrinol Metab*. 5(3): 150-9.

Mortensen M.B., Kulenovic I. and Falk E., (2016). Statin use and cardiovascular risk factors in diabetic patients developing a first myocardial infarction. *Cardiovascular diabetology*. 15(1): 81.

Nakajima K. (2010). Pharmacotherapy of mixed dyslipidemia in the metabolic syndrome. *Curr Clin Pharmacol*. 5(2):133-9.

Nelson F., Fisher M. and McKay G. (2008). Ezetimibe. *Pract Diab Int*. 25(4): 149-150.

Noel Z.R. and Beavers, C.J., (2017). Proprotein convertase subtilisin/kexin type 9 inhibitors: a brief overview. *The American journal of medicine*, 130(2): 229-e1.

Noor M., Waheed A., Muhammad I. & Bakhtiar S. (2016). Is co q10 really valuable in shielding statin induced myopathy-an exploration? *Pakistan Armed Forces Med J*. 66(4): 525-29.

Ortega-Alonso A., Stephens C., Lucena M. I. and Andrade R. J. (2016). Case Characterization, Clinical Features and Risk Factors in Drug-Induced Liver Injury. *Int. J. Mol. Sci*. 17(5): 714.

O'Sullivan S., (2007). Statins: A review of benefits and risks. *TSMJ*. 8: 52-56.

Oudman I. (2013). Creatine kinase and blood pressure: *Clinical and therapeutic implications*. 5(3): 212-216

Ouchi N., Parker J.L., Lugus J. J. and Walsh K. (2011). Adipokines in inflammation and metabolic disease. *Nat Rev Immunol,* 11(2): 85–97.

Padda M. S., Sanchez M., Akhtar A.J., and Boyer J. L. (2011). Drug induced cholestasis. *Hepatology*. 53(4): 1377–1387.

Page M.M. and Watts G.F. (2016). PCSK9 inhibitors – mechanisms of action. *expermet clin pharmacol*. 39(5): 164-7.

Papatheodorou K., Papazoglou D., Papanas N. and Edmonds M. (2016). Complications of Diabetes 2016. *Journal of Diabetes Research.*

Parker B. A., Capizzi J. A., Grimaldi A. S., Clarkson P. M., Cole S.M., Keadle J., Chipkin S., Pescatello L. S., Simpson K., White C. M. and Thompson P. D. (2013). Effect of Statins on Skeletal Muscle *Function. Circulation*. 127: 96-103.

Pedro-Botet J., Millán Núñez-Cortés J., Chillarón J.J., Flores-Le Roux J.A. and Rius J. (2016). Severity of statin-induced adverse effects on muscle and associated conditions: data from the DAMA study. *Expert Opinion on Drug Safety.* 15(12): 1583-1587.

Phan B. A. P., Dayspring T. D., & Toth P. P. (2012). Ezetimibe therapy: mechanism of action and clinical update. *Vascular Health and Risk Management*. 8: 415–427.

Purohit A., Sharma N. and Gaur A. (2017). Association of HA1C with dyslipidemia in Diabetes Mellitus. Sch.J.App.Med. Sci, 5(7E), 2892-2897.

Rabar S., Harker M., O'flynn N. and Wierzbicki A.S., (2014). Lipid modification and cardiovascular risk assessment for the primary and secondary prevention of cardiovascular disease: summary of updated NICE guidance. *BMJ: British Medical Journal* (Online). 349.

Rosenson R. S., Baker S. K., Jacobson T. A., Kopecky S. L. and Parke B. A. (2014). An assessment by the Statin Muscle Safety Task Force: 2014 update. *J Clin Lipido.* 8(3S): S58-S71.

Rotella C.M., Pala L. and Mannucci E., (2013). Role of insulin in the type 2 diabetes therapy: past, present and future. *International journal of endocrinology and metabolism*. *11*(3): 137.

Ruhl C.E. and Everhart J.E., (2012). Upper limits of normal for alanine aminotransferase activity in the United States population. *Hepatology*. 55(2): 447-454.

Russmann S., Kullak-Ublick G.A. and Grattagliano I. (2009). Current concepts of mechanisms in drug-induced hepatotoxicity. *Curr Med Chem*. 16(23): 3041-3053.

Russo M.W., Hoofnagle J.H., Gu, J., Fontana R.J., Barnhart H., Kleiner D.E., Chalasani N. and Bonkovsky H.L., (2014). Spectrum of statin hepatotoxicity: Experience of the drug-induced liver injury network. *Hepatology*. 60(2): 679-686.

Rutishauser J., (2006). The role of statins in clinical medicine-LDL-cholesterol lowering and beyond. *Swiss medical weekly*. 136(3/4): 41-49.

Seuring T., Archangelidi O. and Suhrcke M. (2015). The Economic Costs of Type 2 Diabetes: A Global Systematic Review. *PharmacoEconomics*. 33: 811–831.

Sharma N., Ooi, J.L., Ong J. and Newman D., (2015). The use of fenofibrate in the management of patients with diabetic retinopathy: an evidence-based review. *Australian family physician*. 44(6): 367-370.

Singh D, Cho WC and Upadhyay G (2016). Drug-Induced Liver Toxicity and Prevention by Herbal Antioxidants: An Overview. *Front. Physiol*. 6:363.

Smilkstein, M.J., Knapp G.L., Kulig K.W., Rumack B.H., Prescott L.F., Illingworth R.N., Critchley J.A., Stewart M.J., Adam R.D., Proudfoot A.T. and Keays R., (2003). Drug-induced hepatotoxicity. *N Engl J Med*. 349: 1974-1976.

Soko N., Dandara C., Ramesar R., Kadzirange G. and Masimirembwa C. (2016). Pharmacokinetics of rosuvastatin in 30 healthy Zimbabwean individuals of African ancestry. *Br J Clin Pharmacol*. 82: 326–328.

Stancu C. & Sima A. (2001). Statins: mechanism of action and effects. *J.Cell.Mol.Med*. 5(4): 378-387.

Stroes E.S., Thompson P.D., Corsini A., Vladutiu G.D., Raal F.J., Ray K.K., Roden M., Stein E., Tokgözoğlu L., Nordestgaard B.G. and Bruckert E., (2015). Statin-associated muscle symptoms: impact on statin therapy—European Atherosclerosis Society Consensus Panel statement on assessment, aetiology and management. *Eur heart J*. 36(17): 1012-1022.

Takata K., Psaltis P.J., and Nicholls S.J (2017). Investigate the long term legacy of statin therapy. *J Thorac Dis.* 9(4): 936-939

Tenenbaum A., Fisman E., Motro M. and Adler Y., (2008). Optimal management of combined dyslipidemia: what have we behind statins monotherapy? *In Cardiovascular Diabetology: Clinical, Metabolic and Inflammatory. Facets.* 45: 127-153. Karger Publishers.

Teschke R. (2012). Hepatotoxicity associated with statins. *News of Hepatol*. 11(3): 418-420.

Tesfaye T., Shikur B., Shimels T. and Firdu N. (2016). Prevalence and factors associated with diabetes mellitus and impaired fasting glucose level among members of federal police commission residing in Addis Ababa, Ethiopia. *BMC Endocrine Disorders.* 16: 68.

Thambiah S.C., Samsudin I.N., George E., Zahari S.S., Lee H.M., Muhamad M.A., Hussei Z., Mohd N.N. and Mohamad M., (2016). Relationship between dyslipidaemia and glycaemic status in patients with type 2 diabetes mellitus. *The Malaysian J pathol.* 38(2): 123-130.

Thapa B.R. and Walia A., 2007. Liver function tests and their interpretation. *The Indian J Pediatrics.* 74(7): 663-671.

Thapar M., Russo M.W. and Bonkovsky H.L., (2013). Statins and liver injury. *Gastroenterology & hepatology*. 9(9): 605.

Tokinaga K., Oeda T., Suzuki Y. and Matsushima Y. (2006). HMG-CoA Reductase Inhibitors (statins) might cause high elevation of creatine phosphokinase (CPK) in patients with unnoticed hypothyroidism. *Endo. J.* 53(3): 401-405

Trentman T.L. Avey S.G. and Ramakrishna H. (2016). Current and emerging treatments for hypercholesterolemia: A focus on statins and proprotein convertase subtilisin/kexin Type 9 inhibitors for perioperative clinicians. *J Anaesthesiol Clin Pharmacol*. 32: 440-5.

Unnikrishnan D. and Satish B. (2005). Exertion induced rhabdomyolysis in patients on statin therapy. Nephrology dialysis transplantation. 20(1): 244

Vergès B. (2015). Pathophysiology of diabetic dyslipidaemia: where are we? *Diabetologia*. 58(5): 886–899

World Health Organization, (2016). Global report on diabetes. France. http://www.who.int. Accessed on March 24, 2017.

Wu L. & Parhofer K.G., (2014). Diabetic dyslipidemia. *Metabolism clinical and experimental*. 63: 1469– 1479

Annex One.

Subject Information sheet (English version):

Principal Investigator: **MEZGEBU LEGESSE**

Addis Ababa University

College of health science

Department of medical biochemistry

Dear participant! Here, I the undersigned, at Addis Ababa University College of Health Science, Department of medical biochemistry, graduate Study Program, currently undertaking a research on a topic entitled as, 'comparison of liver enzymes, creatine kinase and lactate dehydrognase of T2DM patients who are on statin at different time period attending diabetic clinic of Tikur Anbesa Specialized Hospital.' For this study, you will be selected as a participant and before getting your consent, you need to know all necessary information related to the study whose detail is explained as follows.

Introduction

Privacy is the state of being free from intrusion, and in the context of health care it concerns the responsibility of a care provider to protect a client from any disclosure to third party (i.e., discovery by others), even unintentional, of personal health data, by providing security to the patient and the patient's records. Confidentiality, in contrast, is the limiting of information to only those for whom it is appropriate. Therefore, this information sheet briefly provides the necessary guide to be considered during the study.

Objective: the main aim of this study is to assess serum level of lipid profile, liver enzymes, creatine kinase and lactate dehydrognase at different period of T2DM patients who are on statin attending diabetic clinic of Tikur Anbesa Specialized Hospital during the study period.

Participants to be included: all T2DM patients who are on statin therapy and T2DM patients who are not starting statin through convenient sampling will be included in the study.

Risks and discomfort: Participant in this project will not cause more discomfort and no need of extra sample other than sample taken for diagnostic purpose. But, there could be minor pain and challenge in color of your skin following the blood drawing. The amount of blood taken from each volunteer throughout the study period is 6ml which will not affect your health. There

is no major risk in participating in this research, as the whole procedure is carried out by physician and /or health professionals following the standard good clinical practice.

Benefits:-There is no immediate benefit from participating in this study. However, you will have the chance to know your serum level of lipid profile, liver enzymes, creatine kinase and lactate dehydrognase from the laboratory result. And if your result reveals any incidental health problems that need immediate treatment, you will be referred to an appropriate health facility. In addition, your participation will contribute in improving the health delivery system forT2DM patients.

Incentive:-There is no financial or material incentive for participating in this study.

Confidentiality: The information that we will collected from this research project will be kept confidential. Information about you that will be collected from the study will be stored in a file, which will not have your name on it, but a code number assigned to it. Which number belongs to which name will be kept under lock and key, and it will not be revealed to anyone except the principal investigator**.**

Participant Rights

Your participation is entirely voluntary and up to you to decide. There is no penalty if you do not agree to participate. Also you have the right not to answer any questions you do not want to. You may also withdraw from the study at any time. If in the middle you decide to stop filling questions and no longer participate, you can stop without worry.

Persons to contact:

If you have any question, you can ask at any time. If you have additional questions about the study, you can contact the:

Principal investigator: MEZGEBU LEGESSE cell phone-0934095576, E-mail mezgebulegesse@gmail.com Thank you for your cooperation.

If you are voluntary to participate in the study we kindly request you to provide your response for the questionnaire in the next page.

Subject Information sheet (Amharic version):

የተሳታፊዎች የፈቃደኝነትና መተማመኛ መረጃ መስጫ ቅፅ

በአዲስ አበባ ዩኒቨርሲቲ የጤና ሳይንስ ኮሌጅ የሕክምና ባዮኬሚስትሪ ትምሕርት ክፍል፣

እኔ መዝገቡ ለገሰ በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የህክምና ባዮኬሚስትሪ የድህረ ምረቃ ተማሪ ስሆን የመመረቂያ ጽሁፌን በጥቁር አንበሳ ስፔሻላይዝድ ሆስፒታል በስኳር ህሙማን ክትትል ክፍል ውስጥ comparison of liver enzymes, creatine kinase and lactate dehydrognase at different period of T2DM patients who are on statin attending diabetic clinic of Tikur Anbesa Specialized Hospital በሚል ርእስ በመስራት ላይ ነኝ፡፡ ለዚህ ጥናት ደግሞ እርስዎ የተመረጡ ስለሆነ ከዚህ ቀጥሎ የሚገኘዉን መረጃ አንብበዉ በጥናቱ ላይ መስማማትዎን ወይም አለመስማማትዎን እንዲያረጋግጡ በትህትና እጠይቃለሁ፡፡

<u>መግቢያ</u>፡- ጥናቱ ከእርሶ የሚወስዳቸዉ ማንኛዉም መረጃወች ሚስጥራዊነት ሙሉ በሙሉ የተጠበቀ ሲሆን እርሶ በጥናቱ አለመሳተፍም ሆነ በማንኛዉም ሰአት ተሳትፎዎን ማቋረጥ ይችላሉ፡፡

<u>የጥናቱ አላማ</u>፡- የትናቱ ዋና አላማ በጥቁር አንበሳ ስፔሻላይዝድ ሆስፒታል የደረጃ ሁለት የስኳር ሁመምተኛ የስብ መቀነሻ መድሀኒት (statin) ከሚወስዱት liver enzymes, creatine kinase and lactate dehydrognase መጠን መለካት እና ማወዳደር ነዉ፡፡ የጥናቱ ዉጤት ለስኳር ህሙማን ጤና እንክብካቤ የሚጠቅም ሲሆን ከዚህም በተጨማሪ እርስዎም ከላይ የተጠቀሱትን ነገሮች እንዲያውቁ ይረዳዎታል፡፡ በጥናቱ ላይ የሚያደርጉት ተሳትፎ ሙሉ በሙሉ በእርሶ በጎ ፈቃደኝነት ላይ የተመሰረተ ነዉ፡፡

በጥናቱ ለመሳተፍ ፍቃደኛ ሲሆኑ ለናሙና ይሆን ዘንድ አምስት ሚሊ ሊትር ያህል ደም በሆስፒታሉ የጤና ባለሙያዎች አማካኝነት የሚሰጡ ሲሆን ናሙና በሚሰጡበት ጊዜ ሁልጊዜ ለምርመራ ከሚሰጡት የተለየ ህመም እና አለመመቸት የለዉም፡፡

ከጥናቱ ጋር በተያያዘ ጥያቄ ቢኖርዎ ወይም ችግር ቢያጋጥሞ በማንኛዉም ሰአት በሞባይል ቁጥር **<u>0934095576</u>** መዝገቡ ለገሰ ብለዉ ይደዉሉ ወይም በኢሜል አድራሻ **<u>mezgebulegesse@gmail.com</u>** መላክ ይችላሉ፡፡

በጥናቱ ለመሳተፍ ፈቃደኛ ከሆኑ እባክዎ ከዚህ ቀጥሎ ባለዉ የስምምነት ቅጽ ላይ በመፈረም ይተባበሩ፡፡

እናመሰግናለን!!!

Annex Two.

Consent form English version

In undersigning the aim of the project, I am giving my consent to participate in the study entitled as, 'comparison of serum lipid profiles, liver enzymes, creatine kinase and lactate dehydrognase at different period of T2DM patients who are on statin attending diabetic clinic of Tikur Anbesa Specialized Hospital Addis Ababa, Ethiopia. I have been informed that the purpose of this study is to assess serum level of liver enzymes, creatine kinase and lactate dehydrognase and associated risk factors at different period of T2DM patients who are on statin attending diabetic clinic of Tikur Anbesa Specialized Hospital Addis Ababa, Ethiopia, I have understood that participation in this study is entirely voluntarily. I have been told that my answers to the questions will not be disclosed to anyone else and no reports of this study never identify me in any way. I have also been informed that my participation or non-participation or my refusal to answer questions will have no effect on me. I understood that participation in this study does not involve risks. I understood that Mezgebu legesse is the contact person if I have any question about the study or about my rights as a study participant.

Respondent's signature_____

Interviewer Name _____ Signature _____Date_____

Informed consent form (Amharic Version)

የፈቃደኝነት ማረጋገጫ ቅጽ

የምርምር ጥናቱ ክፍል የሆኑ መረጃዎችና ሂደቶች ከተብራሩልኝ በኋላ comparison of liver enzymes, creatine kinase and lactate dehydrognase at different period of T2DM patients who are on statin attending diabetic clinic of Tikur Anbesa Specialized Hospital Addis Ababa, Ethiopia በሚል ርዕስ የደረጃ ሁለት የስኳር ህመማን ሆነው የመጥፎ ስብ ክምችትን መቀነሻ መድኀኒት በሚወሰዱት ላይ የጉበትና የጡንቻን ሁኔታ እና ተዛማጅነት ያላቸውን ጉዳዮች ለማጥናት በተዘጋጀው ጥናታዊ ፅሁፍ ለመሳተፍ ሙሉ ፈቃደኝነቴን አሳይቻለሁ፡፡ እኔም በተብራራልኝ መንገድ ተረድቻለሁ፡፡ ምርምሩ ምንም የተለየ የገንዘብ ጥቅማ ጥቅም የሌለው፣ አደጋ የማያስከትል መሆኑን እንዲሁም የሚደርገው ተሳትፎ እና መረጃ በሚስጢር የሚያዝና ለማንም ተላልፎ የማይሰጥ መሆኑን ተረድቻለሁ፡፡ ስለዚህ በዚህ የምርምር ጥናት ላይ ለመሳተፍ ፈቃደኛ መሆኔን በፊርማዬ አረጋግጣለሁ፡፡

የተሳታፊው ፊርማ ----------

የመረጃ ሰብሳቢው ስም---------- ---------ፊርማ----------ቀን ----------

Annex Three

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 and muscle function among type 2 diabetic patients who are receiving statin 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.

3.1. Questioner in English

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

Patient Code; …….

1. Gender: A. Female B. Male
2. Age in year: _____
3. Address of the participant. A. Addis Ababa B. Oromia C. Amhara C. Tigray D. Southern Nation and Nationalities E. Others
4. Education: A) illiterate B) up to high school C) diploma and above
5. Occupation : _____
6. Monthly income in birr: _____
7. Alcoholic intake status: A) drunker B) non drunker
8. If you are drunker please fill the following table

Type of alcohol you drink	Quantities of alcohol you drink		
	Per day	Per week	Per month
Beer in bottle			
Wine in glass			
Hooch in unit			

9. Smoking status: A) currently smoker, _____ cigarette per day B) quitted smoking, last smoked and number cigarette you were smoking per day before quitting _____ C) never smoking D) missing information

10. How many days you have missed taking statin drugs within the last one months?

A. Never B. once C. twice D. three times E. more than three times

Part 2: Health information: please make a circle" on the options that you choose

1. Do you have any liver disease before? A) Yes B) no
2. If your answer is yes to the above question, when? _____-
3. Do you have any muscular injury, trauma or other? A) Yes B) no
4. If your answer is yes to the above question, when? _____
5. Have you taken any other drugs within the last three months? A) Yes B) NO
6. If your answer is yes to the above question list them: _____
7. Have you done HIV tests before? A) yes B) no
8. When your answer for the above question is yes and the result was positive, are you on anti-HIV drug? A) Yes B) no
9. Are you on anti-glycemic drugs? A. yes B. no,
10. If your answer is yes to the above question, since when? _____
11. Type of anti-glycemic drugs? A. Metformin B. sulfonylureas C. thiazolidinedione D. insulin E. combination
12. Are you on statin drugs? A. yes B. no .
13. If your answer is yes to the above questions, since when? _____
14. Dose of statin you are on. A. 20mg B. 40mg C.80mg
15. Type of statin drugs you are on: A. simvastatin B. atorvastatin C. lovastatin D. fluvastatin E. other

Part III: physical and clinical information

1. Height _____
2. Weight _____
3. Hip _____
4. Waist ----------
5. Blood pressure ----------

3.2. **Questionnaire in Amharic**

ለክቡራን ተሳታፊዎች ከዚህ በታች የተዘረዘሩት ጥያቄዎች ከእናንተ የሚፈለጉ ናቸው፡፡ ጥያቄው በአዲስ አበባ ዩኒቨርስቲ ለድህረ ምረቃ ጥናት የሚያስፈልጉ ናቸው፡፡ የዚህ ጥናት አላማ የደረጃ ሁለት የስኳር ታማሚ ሆኖ የመጥፎ ስብ ክምችትን ለመቀነስ መድሐኒት (statin) የሚወስዱ ሰዎችን የጉበታቸውንና የጡንቻቸውን ሁኔታ ማጥናት ነው፡፡ የእናንተ መልስ መስጠት ለዚህ ጥናት አላማ ዕውን መሆን አስፈላጊ ነው፡፡ ለትብብርዎ እናመሰግናለን!!!

ክፍል አንድ

ግላዊ መረጃ ፡- ከዚህ በታች እርስዎን በትክክል የሚገልጽዎትን ክብ ያድርጉ

የተሳታፊው ሚስጥራዊ ቁጥር፡- .....

1. ፆታ ፡- ሀ. ወንድ ለ. ሴት
2. ዕድሜ በዓመት _____
3. አድራሻ፡ ሀ. አዲስ አበባ ለ. ኦሮሚያ ሐ. አማራ መ. ደቡብ ህዝቦች ሠ. ትግራይ ረ. ሌሎች
4. የትምህርት ደረጃ: ሀ. የቀለም ት/ት ያልተማሩ ለ. እስከ መሰናዶ የተማሩ ሐ. ዲፕሎም ና ከዛ በላይ የተማሩ
5. የተሰማሩበት የስራ መስክ _____
6. የወር ገቢዎ _____ የኢትዮጵያ ብር
7. የመጠጥ ሁኔታ፡- ሀ. እጠጣለሁ ለ. አልጠጣም
8. የሚጠጡ ከሆነ እባክዎ የሚከተለውን ሠንጠረዥ ይሙሉት

የሚጠጡት የአልኮል መጠጥ ዓይነት	የሚጠጡት የአልኮል መጠጥ መጠን		
	በቀን	በሳምንት	በወር
ቢራ በጠርሙስ			
ወይን በብርጭቆ			
አስካሪ መጠጥ በመለኪያ			

9. የማጨስ ሁኔታ ሀ. በአሁኑ ጊዜ _____ሲጋራ በቀን/በሳምንት/በወር አጨሳለሁ. ለ. አሁን አቁሜያለሁ፣ መቼ አቆሙ እንዲሁም ከማቆምዎ ብፊት በቀን ምን ያህል ሲጋራ ያጨሱ ነበር _____ ሐ. በጭራሽ አላጨስም
10. ባለፈው አንድ ወር ውስጥ ለምን ያክል ቀን የመጥፎ ስብ ክምችት መቀነሻ መድሀኔት (statin) መውሰደ ረስተዋል? ሀ. በጨራሽ ለ. አንድ ጊዜ ሐ. ሁለት ጊዜ መ. ሦስት ጊዜ ሠ. ከሦስት ቀን በላይ

ክፍል ሁለት

የጤና መረጃ ፡- ከዚህ በታች እርስዎን በትክክል በሚገልፅ ላይ ክብ ያድርጉ

1. ከዚህ በፊት የጉበት በሽታ ችግር አለብዎት ? ሀ. አዎ ለ. የለም

2. ከላይ ለቀረበው ጥያቄ አዎ ከሆነ መልስዎ መቼ? _____
3. ከዚህ በፊት የጡንቻ ወይም የልብ ህመም ነበረብዎት? ሀ. አዎ ለ. የለም
4. ከላይ ለቀረበው ጥያቄ አዎ ከሆነ መልስዎ መቼ? _____
5. ላለፉት ሶስት ወራት የወሰዱት መድሀኒት አለ? ሀ. አዎ ለ. የለም
6. ከላይ ለቀረበው ጥያቄ አዎ ከሆነ መልስዎ መድሀኔቱ ይገለጽ _____
7. ከዚህ በፊት HIV ምርመራ አድርገው ያውቃሉ? ሀ. አዎ ለ. የለም
8. ከላይ ለቀረበው ጥያቄ መልስዎአዎ ሆኖ ውጤቱ ፖዘቲቭ ከሆነ መድሐኒት ዕየወሰዱ ነው ? ሀ. አዎ ለ. የለም
9. የስኳር መቆጣጠሪያ መድሀኒት እየወሰዱ ነው? ሀ. አዎ ለ. የለም
10. ከላይ ለቀረበው ጥያቄመልስዎ አዎ ከሆነ ከመቼ ጀምሮ? _____
11. የሚወስዱት የስኳር መቆጣጠሪያ የመድሀኔቱን ዓይነት A. Metformin B. sulfonylureas C. thiazolidinedione D. insulin E. combination
12. የመጥፎ ስብ ክምችትን መቀነሻ መድሐኒት (statin) እየወሰዱ ነው ሀ. አዎ ለ. የለም
13. ከላይ ለቀረበው ጥያቄ መልስዎ አዎ ከሆነ ከመቼ ጀምሮ? _____
14. የstatin መጠኑ (dose) _____ A. 20mg B. 40mg C.80mg
15. Statin ዓይነቱን A. simvastatin B. atorvastatin C. lovastatin D. fluvastatin E. other

ክፍል ሶስት፡ አካላዊ ምርመራን በተመለከተ

ı. የደም ግፊት ልኬት ----------
2. ቁመት _____
3. ክብደት _____
4. ወገብ ----------
5. ዳሌ ----------

Declaration

I, the undersigned, hereby declare that this MSc thesis is my original work and has not been presented for a degree in any university, and that all sources of materials used for the thesis have been duly acknowledged.

Name of candidate:	signature	date
_____	_____	_____

Name of Advisors:

1._____	_____	_____
2._____	_____	_____
3._____	_____	_____
4. _____	_____	_____