ADDIS ABABA UNIVERSITY
SCHOOL OF MEDICINE
DEPARTMENT OF BIOCHEMISTRY
SCHOOL OF GRADUATE STUDIES

COMPARATIVE STUDY BETWEEN DPP-4 INHIBITORS BASED THERAPIES AND OTHER NON-INSULIN BASED THERAPIES AMONG TYPE 2 DIABETES PATIENTS IN ADDIS ABABA, ETHIOPIA

BY: ALIAS TESFAMARIAM

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

ADDIS ABABA, ETHIOPIA

May, 2016

COMPARATIVE STUDY BETWEEN DPP-4 INHIBITORS BASED THERAPIES AND OTHER NON-INSULIN THERAPIES AMONG TYPE 2 DIABETES PATIENTS IN ADDIS ABABA, ETHIOPIA

MSc THESIS

BY: ALIAS TESFAMARIAM (BSc)

A thesis submitted to the School of graduate studies of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Science in Medical Biochemistry

Major Advisor

Dr. Solomon Genet (PhD), Addis Ababa University, School of Medicine

Department of Biochemistry

Co- Advisors

Dr. Tedla Kebede (MD), Addis Ababa University, School of Medicine

Department of Internal Medicine, Endocrinology unit

Dr. Menakath Menon (PhD), Addis Ababa University, School of Medicine,

Department of Biochemistry

Addis Ababa, Ethiopia

May, 2016

Addis Ababa University

School of Graduate studies

This is to certify that the thesis prepared by **Alias Tesfamariam** entitled: "**Comparative study between DPP-4 inhibitors based therapies and other non-insulin therapies among type-2 diabetes patients in Addis Ababa, Ethiopia**" and submitted in partial fulfillment of the requirements for the Degree of Master of Science in Medical Biochemistry complies with regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

Examiner:

Dr. Helen Yfter (MD) Signature: _____ Date: _____

Advisors:

Dr. Solomon Genet (Advisor) Signature: _____ Date: _____

Dr. Tedla Kebede (Co-advisor) Signature: _____ Date: _____

Dr. Menakath Menon (Co-advisor) Signature: _____ Date: _____

Table of contents

Page

ACKNOWLEDGEMENT

Foremost I would like to thank Almighty God for his indescribable help and judgments throughout my life and throughout this thesis work. My sincere gratitude extends to my major advisor Dr. Solomon Genet for his consistent guidance, encouragement, introducing me to the topic, critical remarks, insightful comments and suggestions and for his engagement throughout this work. God may richly bless him and his family.

I would like to extend my sincere thanks to my co-advisor Dr. Menakath Menon for his hospitality, kindness, cooperation, fruitful discussion, valuable comment, support, constructive guidance, and for all his support in realizing of this paper. It is my honor to express my heartfelt gratitude to my co- advisor Dr. Tedla Kebede for his cooperation in sample collection.

I am also indebted to Mr. Getahun, Mr. Bula, Sister Wubit, Tsion, Rahel and Deset. I would like to convey my indebtedness and heartfelt thanks to all of them who have shared with me all the tremendous ups and downs that I faced through sample collection and laboratory analysis.

I also wish to thank the post graduate office of Addis Ababa University for the financial and material support and Aksum University for covering my living expense throughout my study period.

Last but not least, my sincere thanks also goes to my beloved family: my mother and father, I couldn't have imagined having a better family caring and mentoring for the rest of my life. I also thank my brother Dr. Aregay and his wife Senayt Assefa for their unrestricted support and encouragement throughout my education.

List of Figures

List of Tables

List of Abbreviations and Acronyms

ADA	American Diabetes Association
BMI	Body Mass Index
ACCORD	Action to control Cardiovascular Risk in Diabetes
DKA	Diabetic ketoacidosis
DM	Diabetes Mellitus
DPP-4 inhibitors	Dipeptidyl-peptidase-4 inhibiters
FDA	Food and Drug Administration
FBS	Fasting Blood Sugar
HDL-C	High Density Lipoprotein Cholesterol
IDF	International Diabetes Federation
IDDM	Insulin Dependent Diabetes Mellitus
IGT	Impaired Glucose Tolerance
GDM	Gestational Diabetes Mellitus
GIP	Glucose-dependent Insulin tropic Polypeptide
GLP-1	Glucagon-like peptide-1
LDL-C	Low Density Lipoprotein Cholesterol
OHAs	Oral Hypoglycemic Agents
SU	Sulfonylurea
TC	Total Cholesterol
TG	Triglyceride
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
TZD	Thiazolidinediones
WHO	World Health Organization

Abstract

Background: Type-2 diabetes mellitus is characterized by hyperglycemia as a result of defects in insulin secretion or insulin action. Over the years, attempts to regulate diabetics therapeutically through glycemic control have reduced complications and trauma associated with the disease.

Objective: The aim of the present study was to compare the effect of DPP-4 inhibitors based therapies and non-insulin based therapies among type-2 diabetic patients attending diabetic clinics at Tikur Anbessa Specialized Hospital, and Huleshet Higher Private Clinic, Addis Ababa, Ethiopia.

Methods: Hospital based cross-sectional study was conducted between the two types of treatment strategies among type-2 diabetic patients. Sixty type-2 diabetic patients were randomly selected from patients attending diabetic clinic at Tikur Anbessa Specialized Hospital, who were receiving non-insulin based therapies and 30 type-2 diabetic patients who were receiving DPP-4 inhibitors based therapies from Huleshet Higher Private clinic were included in this study.

Results: The mean total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL), serum urea and serum creatinine were significantly lower in type-2 diabetic patients who received DPP-4 inhibitors based therapies than non-insulin based treated type-2 diabetic patients. The mean value of high density lipoprotein (HDL) was also significantly higher as compared to type-2 diabetic patients who were receiving non-insulin based therapies. The mean value of body mass index (BMI) and fasting blood sugar (FBS) were lower in the DPP-4 inhibitors based therapies as compared to non-insulin based treated type-2 diabetic patients but statistically not significant ($p>0.05$). Among diabetic patients, females had higher level of FBS, TG, LDL, serum urea, BMI and lower level of TC, HDL and serum creatinine as compared to males in both groups. However, the differences were not statistically significant ($p>0.05$).

Conclusion: The DPP-4 inhibitors based therapy helps in normalizing lipid profiles, FBS and also better adjustments in kidney clearance pattern. It also as a result helps in adjusting the weight to height ratios and helps to maintain BMI in the normal range.

Keywords: Type-2 diabetes, DPP-4, Lipid profile, Renal function test, Incretin-based therapy, Body mass index.

1. INTRODUCTION

1.1Background

Diabetes mellitus (DM) is a major public health problem world-wide. It is a significant health care challenge of the 21st century. DM is a metabolic endocrine disorder characterized by chronic hyperglycemia, with disturbances of carbohydrate, fat and protein metabolism occurring as a result of deficiencies in insulin secretion, insulin action, or both [IDF,2013]. The effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs. DM with characteristic symptoms such as thirst, polyuria, blurring of vision and weight loss. In its severe forms, ketoacidosis or a non-ketotichyperosmolar state lead to coma and in absence of effective treatment it may lead to death [Alberti & Zimmet 2004].

There are three main types of DM. These are considered to be type 1 diabetes mellitus (T1DM), is an autoimmune disease in which destruction of pancreatic beta cells occurs. This happens most often in children and adolescents. Type 2 diabetes mellitus (T2DM) is a metabolic disorder that results from the body's inability to either make enough insulin or to use insulin properly. It generally occurs in adulthood, though it is increasingly happening in adolescents as well. A third type is gestational diabetes mellitus (GDM), which develops during pregnancies but disappears when pregnancy is over. However, T2DM is the most prevalent, which accounts for 90% to 95% of all cases [ADA, 2001]. All types of diabetes should be treated under a close collaboration between patients and healthcare providers in order to prevent long-term complications such as damage to the eyes, kidney and heart. People with diabetes must be treated to avoid early death [IDF, 2013].

Currently, T2DM is managed by a combination of diet, exercise and pharmacological Therapy. Significantly, in the ACCORD trial, combination therapy using high doses of thiazolidinediones (TZD), sulfonylureas (SU), metformin, and insulin, was associated with an increase in cardiovascular and hypoglycemia. Metformin and TZDs decrease insulin resistance and hepatic glucose output in patients with significant renal or cardiac dysfunction, both of which occur frequently in T2DM [ACCORD, 2008].

The majority of oral hypoglycemic agents (OHAs) are vulnerable to impaired awareness of hypoglycemia with consequent neuroglycopenia and adverse cardiovascular effects, dictating the need for particular caution with therapies that increase the risk of hypoglycemia. A history of severe hypoglycemia in older T2DM patients has been associated with a greater risk of dementia, which increases with the number of hypoglycemic episodes [Whitmer *et al*., 2009].

In recent years, based on currently available information (phase 3 studies designed for drug approval), incretin mimetics and DPP-4 inhibitors have advantages over other anti-diabetic drugs. Novel effects have been found with incretin-based therapies, such as GLP-1 receptor agonists (exenatide & liraglutide in phase 3 trials) and DPP-4 inhibitors (sitagliptin & vildagliptin), which are beneficial to patients and are not found with other anti-diabetic treatments in T2DM. Perhaps the most significant of these is the glucose-dependent nature of their insulinotropic effects, which means that DPP-4 inhibitors based therapies, mimic closely the physiologic insulin profile and are associated with very low rates of hypoglycemia. In addition to this key property and also of major significance clinically is that, DPP-4 inhibitors based therapies do not cause weight gain in T2DM patients [Nauck *et al*., 2011].

1.2. Classification of Diabetes Mellitus

DM is a group of metabolic diseases characterized by elevated blood glucose levels (hyperglycemia) resulting from defects in insulin secretion, insulin action or both. Insulin is a hormone manufactured by the β-cells of the pancreas, which is required to utilize glucose from digested food as energy source. Chronic hyperglycemia is associated with micro vascular and macro vascular complications that can lead to visual impairment, blindness, kidney disease, nerve damage, heart disease and stroke [IDF, 2013]. Generally, diabetes is categorized into three major forms known as diabetes T1DM, T2DM and GDM.

T1DM is not preventable with current knowledge and was previously referred to as insulin dependent, juvenile or childhood-onset diabetes. T1DM is characterized by autoimmune destruction of β –cell of pancreas. The cause of T1DM is still not known. However, the risk for developing T1DM has been linked to exposure to some viral infections or environmental

factors. Also the risk for developing the disease slightly increases, if there is a family member with the disease (IDF 2014). T1DM is characterized by the lack of insulin production by the pancreas and requires daily administration of insulin. Symptoms of T1DM include polyuria (excessive urine), polydipsia (excessive thirst), and weight loss, changes in vision, tiredness and constant hunger. This form of diabetes, accounts for only 5–10% [WHO, 2015a].

The most common form, diabetes T2DM, was in contrast to T1DM used to be called non-insulin dependent diabetes or adult onset diabetes. There is no autoimmune destruction of pancreas present in this form. These individuals do produce insulin, but either it is insufficient or they have developed a resistance towards its effect. Frequently, patients go undiagnosed for several years until negative complications occur. The explanation is that the hyperglycemia is not high enough, during these years, to create significant symptoms. Unfortunately most of the patients with T2DM are obese and overweight itself induce insulin resistance. Other known risk factors for T2DM are poor diet, physical inactivity, advancing age, heredity of diabetes, ethnicity and raised blood glucose during pregnancy affecting the fetus [IDF, 2013].

T2DM, which used to be only seen in adults but can now be diagnosed at any age, even with children, because it may remain undetected for many years. It is not always, but usually it is associated with obese or overweight people with a sedentary life style. Other risk factors that could lead to the development of T2DM include: family history and history of gestational diabetes. It is often diagnosed when complications (e.g. kidney failure, blindness, lower limb amputation, cardiovascular disease) appear, or when a routine blood or urine glucose test is done. During the early stages, T2DM can be managed through healthy diet and regular physical activity but as it progresses, there will be a need for oral drug or insulin [IDF, 2014]. T2DM accounts for 90% to 95% of all diagnosed diabetes cases. This form of diabetes generally begins as insulin resistance and, because the body is unable to produce enough insulin to address the resistance, the pancreas may reduce the production of insulin or eventually stop producing it [ADA, 2001].

GDM occurs to women during pregnancy. It is indicated by hyperglycemia with above normal blood glucose levels but below the diagnostic of diabetes. When this happens, the risk of complications during pregnancy and at delivery is increased. Also the possibility of having diabetes type 2 in the future is higher. It occurs in about 2%–5% of all pregnancies and may improve or disappear after delivery [WHO, 2015a.].

1.3 Pathophysiology of type 2 Diabetes Mellitus

T2DM is complex and comprises a variety of different dysfunctions involving multiple organs and tissue types. In previous understanding, the pathophysiology of T2DM largely focused on β- cell dysfunction and insulin resistance in skeletal muscle and liver, and that understanding has expanded in recent years to include defects in the adipose tissue, pancreatic α -cells, gastrointestinal tract, brain, heart and kidney [Lorenzo *et al*., 2010]. T2DM is a multisystem disease with multiple metabolic abnormalities that contribute in varying degrees to the development and maintenance of hyperglycemia. Chronic hyperglycemia is a primary factor in the pathophysiology of T2DM because of its contribution to the development of insulin resistance and β-cell dysfunction, both of which, and in turn, aggravate hyperglycemia [Herrera *et al*., 2004]. The pathogenesis of T2DM is depicted in (Fig 1).

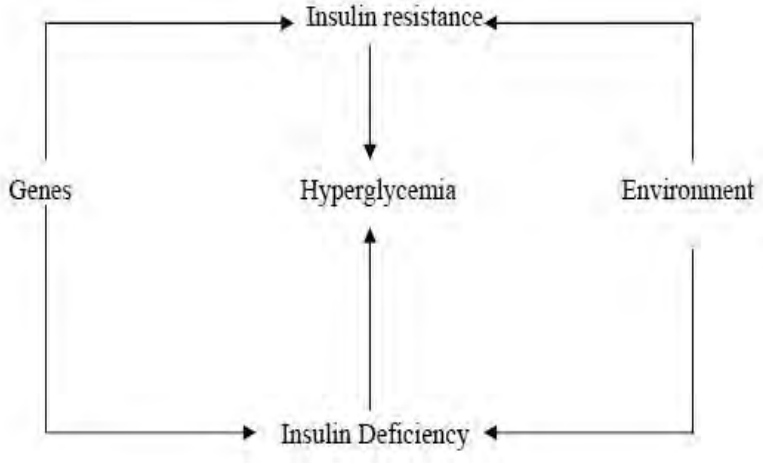


Figure 1: Pathogenesis of type 2 diabetes [Giorgino *et al.,* 2005].

The term insulin resistance characterized by impairment in insulin action in insulin-target tissues such as skeletal muscle, liver, and adipose tissue (fat cells). Insulin is the major pancreatic islet hormone in the body and it regulators of glucose metabolism, stimulates glucose uptake by skeletal muscle, and suppresses hepatic glucose production (HGP), and lipolysis in adipocytes [Neeland *et al*., 2012]. In the presence of insulin resistance, all of these insulin actions are markedly impaired, leading to impair insulin-mediated muscle glucose uptake and increased rates of HGP and lipolysis [Sachin *et al.*, 2009]. In addition to hepatic insulin resistance, multiple other factors contribute to accelerated rate of HGP, including:

- Increased circulating glucagon levels and enhanced hepatic sensitivity to glucagon
- Increased circulation of gluconeogenic precursors such as lactate, alanine and glycerol
- Increased free fatty acid (FFA) oxidation [Ismail, 2012].

1.4 Prevalence of Diabetes Mellitus

In the 21st century non-communicable diseases (NCDs) account for one of the major health and socioeconomic threats [Rudasingwa *et al,* 2012]. In parallel, DM is now one of the most common NCDs globally. It is becoming one of the leading causes of death in the developed world and its prevalence is substantially increasing in the developing countries [IDF, 2013].

DM is a heterogeneous disorder with varying prevalence among different ethnic groups. DM is estimated to affect as many as 408 million people in 2013 worldwide, and is expected to reach up to 776 million people by the year 2035 (IDF, 2013). In 2013, the top 10 countries with higher prevalence of diabetes are Tokelau (37.5%), Federated States of Micronesia (35%), Marshall Islands (34.9%), Kiribati (28.8%), Cook Islands (25.7%), Vanuatu (24%), Saudi Arabia (23.9%), Nauru (23.3%), Kuwait (23.1%) and Qatar (22.9%) [IDF, 2013].

DM is no longer rare in Africa. Africa is the region with the lower prevalence of diabetes (4.9%), having Reunion (15.4%), Seychelles (12.1%) and Gabon (10.7%) as the top three countries with higher prevalence and 10 out of 48 countries with prevalence of diabetes higher than the upper quartile (6.3%) prevalence [IDF, 2013].

According to the 2011 report, the number of adults living with DM in Ethiopia was 3.5% [IDF, 2011]. Evidence shows that DM is claiming the lives of more than 4 million people worldwide annually and developing countries account for a substantially high proportion. The prevalence of T2DM is rapidly increasing worldwide. The most important demographic change to DM prevalence across the world appears to be the increase in the proportion of population >65 years of age [Wild et al., 2004].

1.5 Complications of Diabetes

DM is associated with serious complications, which can be acute or chronic. Acute complications include diabetic ketoacidosis (DKA), which is a medical emergency and caused by excess ketone body production as a result of uncontrolled diabetes [Rask-Madsen & King, 2013]. As a chronic disease, complications of DM may affect the functioning of various organ systems, and account for much of the morbidity and mortality associated with this disease. These chronic complications can be two major categories: vascular and non-vascular. Vascular complications are further divided according to whether they are microvascular (retinopathy, neuropathy, and nephropathy) or macrovascular (coronary artery disease, peripheral arterial disease, and cerebrovascular disease) and Conditions such as infections and skin changes constitute the non-vascular complications of diabetes [IDF, 2014].

The increased risks of vascular and non-vascular diseases are directly associated with the duration of hyperglycemia. Chronic hyperglycemia which is the major cause of microvascular and macrovascular complications [Fowler, 2008]. An increased risk of cardiovascular disease in patients with DM and also reported a two to four-fold increase in coronary artery disease events and mortality in patients with T2DM [Turnbull *et al.,*2009]. A meta-analysis of intensive glycemic control and macrovascular complications in T2DM patients also suggests that a strict lowering of glucose levels reduces the risk of major cardiovascular events (Turnbull *et al.,* 2009). Factors such as dyslipidemia and hypertension also play important roles in the development of the macrovascular complications of T2DM (Fowler, 2008).

1.6 Diagnosis of Diabetes Mellitus

DM can be diagnosed by the presence of four classic signs that include polyuria, polyphagia, polydipsia, and for most hyperglycemia patients [Vasudev & Jann, 2011]. T2DM is characterized by persistent hyperglycemia, and is diagnosed by demonstrating one of the following tests:

1. Fasting blood glucose test (FBG) which is the most common. Fasting blood glucose levels are checked after fasting for between 12 and 14 hours. The normal FPG which is ranging from 70-110mg/dl and the abnormal FBG level is ≥ 126 mg/dl.
2. Random blood glucose test - blood glucose levels are checked at various times during the day. Blood glucose levels tend to stay constant in a person who does not have DM. Random plasma glucose ≥ 200 mg/dl Plus symptoms such as polyuria, polydipsia, and weight loss.
3. Oral glucose tolerance test (OGTT) - a high-glucose drink is given. Blood samples are checked at regular intervals for two hours. A 75 g OGTT with a 2 h value of plasma glucose ≥ 200 mg/dl.
4. Glycoslated hemoglobin (HbA1c) - measures how much glucose is stuck to red blood cells. It also shows how well DM has been controlled in the last 2 to 3 months and whether DM medicine needs to be changed. HbA1c of 6.5% is recommended as the cut point for diagnosing DM. A value of less than 6.5% does not exclude DM, diagnosed using glucose tests [WHO, 2011].

1.7 Management of Diabetes Mellitus

The goals of treatment for DM are to reduce and control blood glucose levels, relieve the symptoms of the disease, and prevent complications. Intensive treatment and careful control of blood glucose levels can reduce the risk of complications from DM [ADA, 2003 a]. The American Diabetes Association recommends the formulation of an individualized DM management plan in collaboration with the patient. A high degree of patient involvement in self-management should be part of this plan, including frequent self- monitoring of blood glucose. Long-term control of blood glucose levels can be assessed by measuring the extent to which glucose is bound to the HA1c component of

hemoglobin. This Glycoslated hemoglobin value is expressed as a percentage and should be less than 7% in patients with DM [ADA, 2003a].

The successful management of DM requires lifestyle changes for the patient such as diet, exercise, self-monitoring of blood and possibly urine. The patient must be involved in the decision making process and must learn as much as possible about diabetes, including why the symptoms of diabetes occur and how they can be alleviated through control of blood glucose levels. Education is essential for motivating patients to manage their disease, encouraging changes in lifestyle, and improving patient outcomes. Disease management programs provide an excellent way to integrate education into the management of the disease [ADA, 2003 b].

1.7.1 Non-pharmacologic Interventions

Medical nutrition therapy (i.e., diet) and exercise are important aspects of non pharmacologic treatment for DM. Weight loss is a vital part of treatment for T2DM because it can help improve the sensitivity of cells to insulin and the uptake of glucose by cells. A goal of medical nutrition therapy is to attain and maintain blood glucose levels in the normal range or as close to normal as possible. Patients with T2DM typically have dyslipidemia, and another goal for these patients is to improve the lipid profile [ADA, 2003 b].

1.7.2 Pharmacologic Interventions

Pharmacological interventions or medication therapy are required in the treatment of T2DM when lifestyle modification alone proves insufficient to improve the glycemic control and reduce the risk of cardiovascular complications. In T2DM, important risk factors include central obesity, age and decreased physical activity among adults [Capriotti, 2005].

The National Institute for Health and Care Excellence (NICE) provides guidelines for T2DM management and states that the first line in drug treatment for T2DM is a class of oral anti-diabetic agents, namely: metformin, insulin secretagodues and acarbos, which is collectively known as oral glucose control therapies. The second line drug treatment for T2DM is other oral agents (e.g. Di-peptidylpeptidase-4 (DPP-4) inhibitor, thiazolidinediones) and Glucagon-Like

Peptide-1 (GLP-1) receptor agonist. These drugs are introduced when the control of blood glucose level remains and / or becomes insufficient, or if the patient does not tolerate the first line drug therapies. The final stage is the initiation of insulin therapies or/ and combining insulin with an oral agent when the oral therapies fail [NICE, 2009].

1.7.2.1 Incretin Based Therapies

Chronic hyperglycemia that characterizes type 2 DM results from the combination of two pathogenic mechanisms that influence each other either by decreased insulin secretion or insulin resistance. In addition, due to damage pancreatic α- cells, there is a high concentration of glucagon, which paradoxically increased after administration of glucose or after ingestion of carbohydrates [Baggio & Drucker, 2007].

Discovery of incretin-based therapies, and its pathogenetic role in T2DM caused an important evolution in pathogenesis and management of diabetes. GLP-1 receptor agonists mimic the effects of endogenous GLP-1, while DPP-4 inhibitors prevent rapid degradation of the hormone. Both GLP-1 receptor agonists and DPP-4 inhibitors minimize the risk of hypoglycemia seen in insulin and some other oral anti-diabetic drugs. Treatment with GLP-1 receptor agonist or a DPP-4 inhibitor could produce an improvement in β-cell function in humans. This would be important to increase β-cell mass and β-cell function is already significantly lower for the risk associated with T2DM diseases [Baggio & Drucker, 2007].

Incretins are gut hormones that increase insulin secretion after meal ingestion in a glucose-dependent manner. GLP-1 and glucose-dependent insulin tropic polypeptide (GIP), two major incretin hormones that are secreted into the circulation by 'L' and 'K' cells of the small intestine, respectively, are responsible for 50% to 70% of glucose dependent insulin release [Baggio & Drucker, 2007]. Apart from insulinotropic effects, GLP-1 also suppresses glucagon release, reduces hepatic gluconeogenesis, delays gastric emptying and reduces food intake by promoting satiety [Zander *et al*., 2002]. The biology of incretin is depicted in (Fig 2)

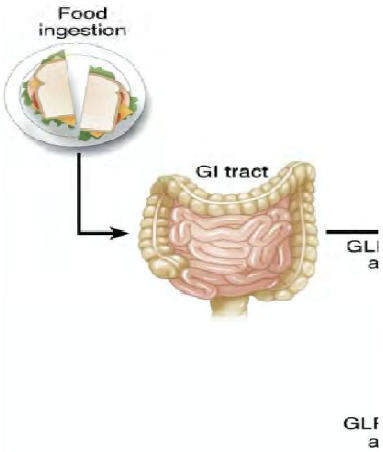


Figure-2: The biology of incretin [Baggio & Drucker, 2007].

1.7.2.1.1 Glucagon-like peptide-1 action (GLP-1)

GLP-1 is cleaved post-translational from pro-glucagon L neuroendocrine cells of the intestinal mucosa and the nervous system, but not in pancreatic α-cells. GLP-1 stimulates the secretion of pancreatic beta cells and also inhibits the α-cell glucagon secretion. These two actions are strictly dependent on glucose production and lead to a normalization of glucose both fasting and postprandial. In terms of hypoglycemia, counter unaffected by glucagon and insulin secretion is stimulated. GLP-1 is therefore able not only to produce hypoglycemia [Nathan *et al.*, 2006].

GLP-1, though found to be the most powerful insulin stimulator, still had poor response on people with T2DM. Therefore, GLP-1 agonists were developed to lengthen and upgrade GLP-1's activity of reducing blood glucose levels.GLP-1 agonists are now licensed for use in T2DM. These agents are injectable therapies, given subcutaneously [Ahren, 2013]. GLP-1 agonist has a wide range of effects among which there are; reduces both fasting and postprandial glucose levels which stimulates the secretion of glucose dependent insulin and suppresses the inappropriate elevation of glucagon; it also leads to weight and systolic blood pressure reduction [Pratley, 2008]. GLP-1 receptor agonists are safe and highly tolerable with the only persistent adverse reaction being nausea and vomiting, which appears mostly only in the early weeks after therapy has begin.

There are now four different types of GLP-1 analogues available: the original exenatide 5-10mg is injected subcutaneously twice daily, the newer longer acting version of exenatide 2 mg is injected once weekly, liraglutide 0.6-1.2 mg and lixisenatide 10-20 mg, are both injected once daily. Both exenatide and liraglutide when used as add on to metformin, sulfonylurea and thiazolidinedione result in reduced HbA1c, fasting and prandial glucose as well as body weight [Ahren, 2013]. The physiological effect of GLP-1 is depicted in (Fig 3).

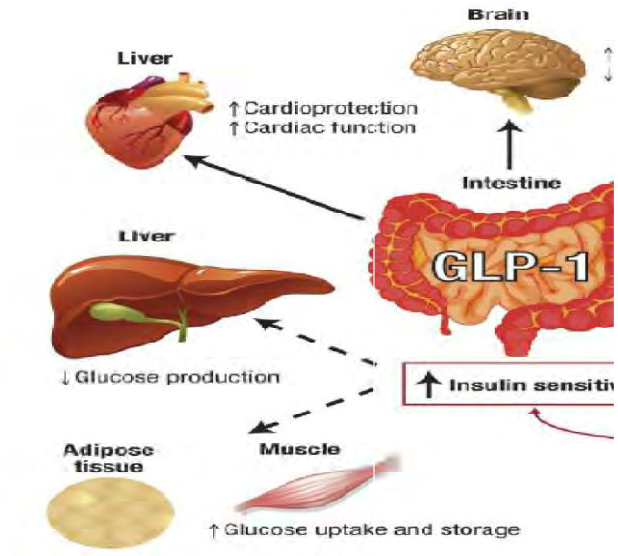


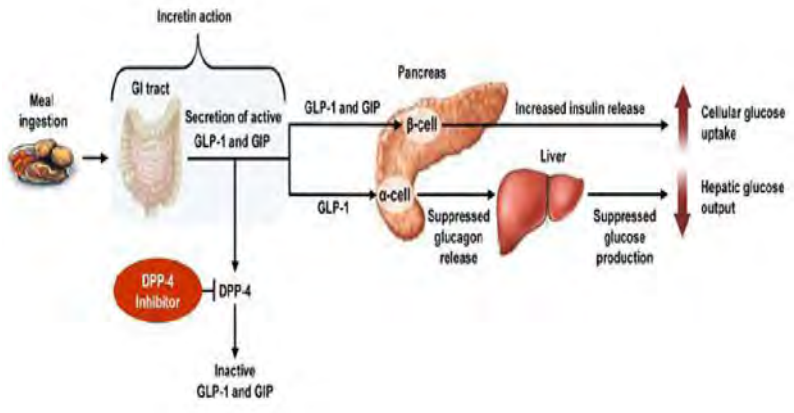
Figure-3: Multiple physiologic effects of glucagon-like peptide-1 (GLP-1) [Baggio &Drucker, 2007].

1.7.2.1.2 Dipeptidylpeptidase-4 (DPP-4) Inhibitor

DPP- 4 is a ubiquitous enzyme that is responsible for the inactivation of both incretin hormones GLP-1 and GIP. DPP -4 inhibitors are FDA approved oral medications in T2DM, which inhibit dipeptidyl peptidase and increase circulating concentrations of incretin hormones and provide glycemic control with improved islet cell function [Pratley & Salsali, 2007]. DPP-4 inhibitors are termed incretin enhancers as they prolong the half-life and availability of endogenous GLP-1 by inhibiting DPP-4.

DPP-4 inhibitors are oral tablets taken once or twice daily, developed to prevent the activity of the DPP-4 enzyme and prolong and strengthen the activities of GLP-1. The first study that showed the possibility of using DPP-4 was tested for a period of four weeks and the result was that it showed a reduction in both prandial and fasting blood glucose as well as HbA1c. DPP-4 inhibitors can be used as a monotherapy, or in combination with metformin, sulfonylurea, thiazolidinedione and insulin [Ahren, 2013].

Regardless of how it is used, there was improvement in glycaemia without weight gain or even with a slight reduction in weight with very low risk for negative effects including hypoglycemia. DPP-4 inhibitors, such as sitagliptin (Januvia), vildagliptin (Galvus), saxagliptin (Onglyza) and linagliptin are now clinically used in a lot of countries by patients [Ahren, 2013]. Sitagliptin was the first DPP-4 inhibitor approved in the U.S.A for clinical use in October 2006 followed by vildagliptin in Europe and saxagliptin in the US markets [Dhillon, 2010]. It exerts its glucoregulatory actions through prevention of incretin degradation, leading to increase of GLP-1 and GIP action. Sitagliptin is administered as a single 100-mg daily tablet either as monotherapy or in combination therapy with oral anti-diabetic agents. Sitagliptin is well tolerated and is not associated with nausea or vomiting as the levels of endogenous intact GLP-1 achieved following DPP-IV inhibition are at the upper limit of the normal physiological range; hence, it is not sufficient to induce an adverse response [Hansotia *et al*., 2004]. The physiological effect of DPP-4 inhibitors is depicted in (Fig 4).



Figur-4: The physiological effect of DPP-4 inhibitors [Pratley & Salsali, 2007].

1.7.2.2 Non- insulin based therapies

The Treatment of diabetes T2DM with oral anti-diabetic agents concentrates on reducing fasting and post meal hyperglycemia as well restoring and maintaining a good glycemic control. The major classes of oral anti-diabetics agents for the treatment of T2DM have different modes of action, safety profiles and tolerability. These classes include agents that increase insulin secretion, increase insulin action, and minimize hepatic glucose production and delay digestion and absorption of intestinal carbohydrates. Oral anti-diabetic agents should be started with a low dose, then after the measurement of Glycoslated hemoglobin (HbA1c) which is done by self-monitoring of capillary blood glucose by some patients, the dose may then be titrated up conforming to the glycemic response. The major classes of oral anti-diabetic agents have usually had a comparable average glucose-lowering effect, thus about an average of 1–2% reduction in HbA1c [Krentz & Bailey, 2005].

A) Sulfonylurea

Sulfonylurea has been largely used for half a century for T2DM treatment. These agents stimulate the secretion of insulin from the β-cell of the pancreatic islet, thereby lowering blood glucose levels. They are very effective when used early after T2DM diagnosis and are also good first line oral drugs for patients who, despite using non pharmacological measures (e.g. diet, exercise), have not achieved or cannot maintain a desired glycemic index and are not over weight. They are cheap and can also used as combination therapy with other anti-diabetic agents. However, they can cause hypoglycemia due to their ability to inhibit glucose production and stimulate insulin release at a low glucose level. They also have a side effect of increased appetite, unwanted weight gain and long term cardiovascular disease risk [Krentz & Bailey, 2005].

B) Biguanides

Metformin is one of the cheapest and most recognizable oral anti-diabetic drugs available for treatment T2DM. Metformin as a foundation of oral therapy in combination with other oral anti-diabetic drugs prescribed for T2DM patients, such as sulfonylurea. Metformin it increases hepatic sensitivity, coordinates cellular energy as well as glucose and lipid metabolism. It exhibits glucose lowering actions without causing overt hypoglycemia and weight gain; in

some cases it enhances weight loss in obese patients. Glucose uptake by the skeletal muscle is also increased by metformin [Capriotti, 2005].

C) Alpha-Glucosidase Inhibitors

The alpha- glucosidase inhibitors available include miglitol, voglibose and acarbose being the most common. They have a good safety record of reducing postprandial hyperglycemia by slowing down the digestion of carbohydrate, without causing weight gain or hypoglycemia. The most common side effect of alpha-glucosidase inhibitor agents is the gastrointestinal agent [Krentz, Patel & Bailey, 2008].

D) Thiazolidinediones

TZD are a class of insulin-sensitizing drugs that are agonists for the nuclear receptor peroxisome proliferator-activated receptor-γ (PPARG). The first TZD, troglitazone (Rezulin©), was approved for use in the United States in 1997, immediately followed by pioglitazone (Actos©) and rosiglitazone (Avandia©).Thiazolidinediones are insulin sensitizers that increase insulin sensitivity and action in liver, muscle, and fatty tissues to endogenous and exogenous insulin [Yki-Jarvinen, 2004].

1.8 Significance of the Study

T2DM is a progressive and complex disorder that is difficult to treat effectively. The majorities of T2DM patients were overweight or obese at diagnosis. The first line oral anti-diabetic drugs were cause weight gain, hypoglycemia and other micro and macro vascular complications either as monotherapy or in combination with other oral anti-diabetic therapy. Today's clinicians are presented with an extensive range of oral anti-diabetic drugs for treatment of T2DM. The main classes are heterogeneous in their modes of action, safety profiles and tolerability. But, recently introduced DPP-4 inhibitors based therapies appear to offer advantages over other oral anti diabetic drugs for T2DM treatment. Because, these DPP-4 inhibitors based therapies have a beneficial effect on glycemic control and weight loss. Therefore, the purpose of this study was to compare effects of the two types of anti-diabetic drugs among type-2 Diabetic patients by using biochemical parameters such as FBS, BMI, lipid profile and renal function tests.

2. OBJECTIVES

2.1 General Objective

- The aim of this study was to assess and compare the effects of DPP-4 inhibitors based therapies and other non-insulin based therapies among type -2 diabetic patients.

2.2 Specific Objectives

- To estimate and compare fasting blood glucose level between DPP-4 inhibitors based therapies and non-insulin based therapies among type -2 diabetic patients.
- To measure and compare lipid profiles of the DPP-4 inhibitors based therapies and non-insulin based therapies among type -2 diabetic patients.
- To assess and compare serum urea and creatinine level between DPP-4 inhibitors based therapies and non-insulin based therapies among type -2 diabetic patients.
- To determine and compare the body mass index (BMI) level between DPP-4 inhibitors based therapies and non-insulin based therapies among type -2 diabetic patients.

3. MATERIALS AND METHODS

3.1 Study Area

The study was conducted in Diabetic center at Tikur Anbesa Specialized Hospital, and Huleshet Private Higher Clinic Addis Ababa, Ethiopia.

3.2 Study Design and Period

A hospital based cross-sectional study was conducted in Addis Ababa, Tikur Anbessa Specialized Hospital and Huleshet Private Higher Clinic from January 2016 to March 2016. In this period, Blood samples were collected by trained medical professionals from type -2 diabetic patients attending at both the clinics.

3.3 Source of population

The source of population was type-2 diabetic patients who were attending diabetic clinics at Tikur Anbessa Specialized Hospital and Huleshet Private Higher Clinic in Addis Ababa.

3.4. Study population

The study population consisted of 60 type-2 diabetic patients who were receiving non-insulin based therapies and 30 type-2 diabetic patients who were receiving incretin-based therapies. Hence a total 90 type-2 diabetic patients were enrolled in the study.

3.5 Sampling technique and Sample Size determination

Convenience sampling technique and simple random sampling was applied among T2DM patients who have been attending Huleshet Private Higher Clinic and Tikure Anbessa Specialized Hospital, Addis Ababa respectively. The sample sizes were estimated by using a single proportion formula and calculated as follows:-

$$\mathbf{n = Z^2pq / d^2}$$

P= assumed the highest population proportion prevalence of diabetes mellitus in Ethiopian adults 4.36%, [IDF.2011]. 5% marginal error (d) to get sample size and Confidence interval (CI) of 95%.

n = Sample size

p = Proportion of DM= 0.0436

d = Margin of error =0.05

q = 1-p =1- 0.0436 =0.9564

Z = 1.96 at 95% Confidence Interval (CI)

$n = \frac{(1.96)^2 \times 0.0436 \times 0.9564}{0.05 \times 0.05} = \frac{0.160191}{0.0025} = 60.0764 = 60$

60 type-2 diabetic patients were randomly recruited for non-insulin based therapies study group and 30 type-2 diabetic patients were recruited for incretin-based therapies for comparison.

3.6 Study variables

3.6.1 Independent variables

- Socio-demographic characteristics
- Duration of diabetes
- Type of drugs
- Alcohol intake status
- Smoking status
- Family history

3.6.2 Dependent variables

- Lipid profile (TC, TG, LDL-C, HDL-C)
- Fasting blood sugar level
- Renal function test (serum urea and creatinine)
- Body Mass Index

3.7 Inclusion and Exclusion Criteria

3.7.1 Inclusion Criteria

- All patients with T2DM
- Both sexes 18 and above years
- Did not suffer from HIV/AIDS
- Did not have malignancies

3.7.2 Exclusion Criteria

- Both sexes male and female patients who were less than 18 years in age.
- Patients who suffer from HIV/AIDS.

- Patients with cancer / malignancies were not included in the study.
- T1DM and any diabetic patients T2DM taking only insulin medication.
- Pregnant women.
- Patients who had refused to participate in the study.
- Not capable of independent communication.
- Patients who were on statin drug treatments.

3.8 Ethical Consideration

Ethical clearance was obtained from Research and Ethical Committee of the Department of Biochemistry, School of Medicine, College of Health Sciences, Addis Ababa University after full review was conducted, and approval was given with protocol number DRERC 07/15 attended by the research committee. A consent form was prepared with a detailed explanation of the objectives, risks, and benefits for the study subject (see Annexs). The confidentiality of the study data was maintained by coding of samples and data. Blood Samples were collected by trained health professionals following standard and ethical procedures.

3.9 Method of Data collection

Collection of patients' data such as demographic characteristics, medical characteristics, and the type of medication used as well as interview by professionals regarding T2DM management were carried out by trained data collectors. Patients' history were collected by reviewing medical charts (data abstraction tool can be found in Annex-I). The demographic data of the patients were collected only after clinical examination by a physician.

3.10 Serum Sample Collection

Five ml of venous blood was drawn from each volunteer patient using a disposable plastic syringe. The blood was poured into a test tube and then centrifuged at 3500 revolution per minute for 10 minutes after it clotted. The serum was transferred to sterile tubes and stored in a deep freezer at minus 70^0C until analysis.

3.11 Data Entry and Analysis

Data was entered and analyzed using SPSS version 20. Categorical variables were presented in numbers and percentages. Continuous variables including demographics, clinical and medication used were presented as complete data sets (mean, standard deviation, minimum and maximum value, and 95% CI). Clinical and laboratory data are expressed as mean ± standard deviation and mean difference was determined using independent sample t- test for comparison between the groups. Within patient comparisons were made using paired two-tailed t- tests and associations were also evaluated by using bivariate analysis with Pearson correlation coefficient. Significance was set at $p<0.05$.

3.12 Biochemical Measurements and Anthropometric Measurements

3.12.1 Body Mass Index

Patient‘s weight was measured using a standard balance, and height was measured using a height measuring device attached to the balance. Body Mass Index (BMI) was calculated from the body weight (kg) and height (meter) as follows:-

$$\text{BMI} = \text{Weight (in kg)} / (\text{Height in m})^2$$

Based on the National Institutes of Health Guidelines on Overweight and Obesity (2014), subjects with BMI below 18.5 kg/m^2 are classified as underweight, BMIs from 18.5 kg/m^2 to 24.9 kg/m^2 as classified as normal, BMIs from 25.0 to 29.9 kg/m2 as classified as overweight, and BMIs at or above 30.0 kg/m^2 are considered obese.

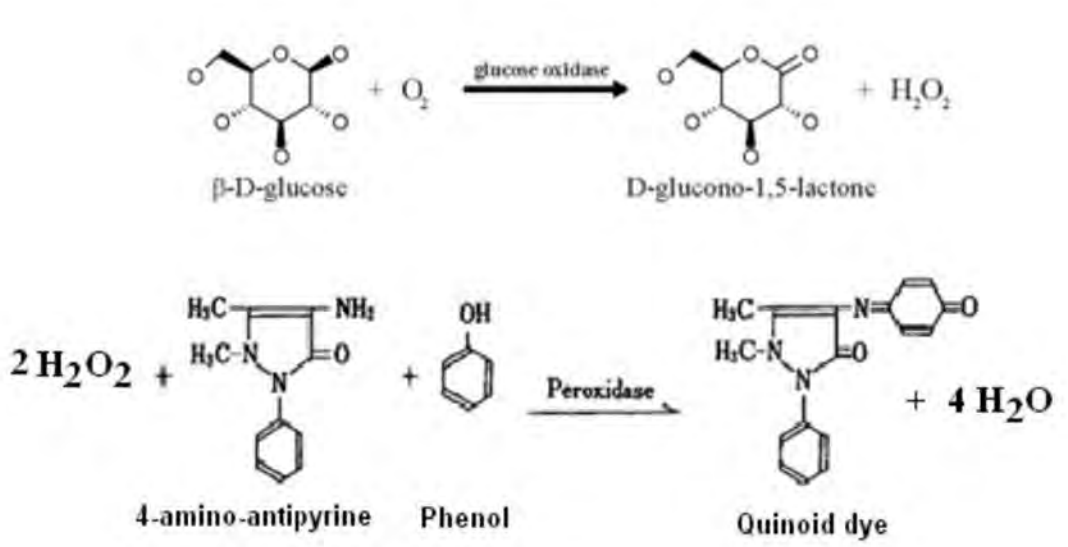
3.12.2 Determination of Serum Fasting Blood Glucose

Glucose levels in the patients’ serum samples were estimated by using a commercial kit based on the method developed by Coxon and Schaffer obtained from Fluitest ® GLU, Germany [National Heart, Lung and Blood Institute,2014].

Principle: Oxidation of glucose by glucose oxidase generates D-gluconolactone plus hydrogen peroxide. The hydrogen peroxide generated then reacts with phenol and 4- aminoantipyrine in the presence of the enzyme, peroxidase to form a colored quinoid dye product. The absorbance

of this colored product is proportional to the glucose concentration in the original sample, and was measured at 546 nm.

Reagents: R1: 150 mmol/L Phosphate buffer pH 7.5, containing 7.5 mmol/l phenol, 12,000 U/l Glucose Oxidase, 660 U/L Peroxidase, 0.40 mmol/l of 4-aminoantipyrine R2: 100 mg/dl (5.55 mmol/l) of glucose standard solution.



Procedure*:* To labeled test tubes, 1.0 ml of reagent R1 was mixed with 10 µl of sample or glucose standard (R2). After 15 minute of incubation at room temperature, the absorbance of sample and standard were measured at 546 nm against reagent blank by using a 5010 spectrophotometer [Coxon & Schaffer, 1971].

Calculation of Glucose concentration

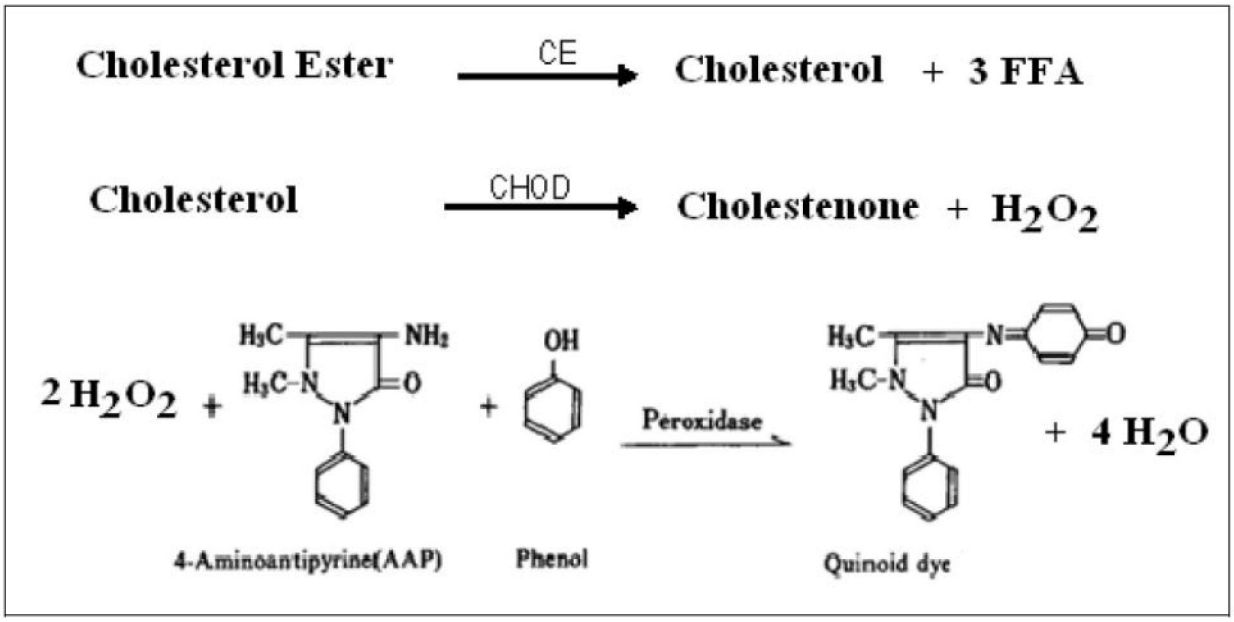
Glucose (mg / dL) = (A sample / A standard) X concentration of the standard Where A is Absorbance at 546nm.

3.12.3 Determination of Serum Total Cholesterol Level (TC)

Serum total cholesterol was estimated by using a commercial kit, based on the method developed [Coxon & Schaffer, 1971].

Principle: The method for the measurement of serum total cholesterol involves the use of three enzymes: cholesterol esterase (CE), cholesterol oxidase (CHOD) and peroxidase (POD).

Cholesterol esters are first hydrolyzed to release free cholesterol and triglycerides using cholesterol esterase. The free cholesterol is then oxidized by CHOD to generate H2O2. The hydrogen peroxide reacts with phenol and 4-aminoantipyrine in the presence of peroxidase to generate a colored quinoid dye product, the absorbance of which is measured at 546 nm, and is proportional to the concentration of total cholesterol in the original sample.



Reagents: R1: 200 mmol/L PIPES pH 7.0, containing 1 mmol/L sodium cholate, > 250 U/L cholesterol esterase, >250 U/L cholesterol oxidase, > 1 KU/L peroxidase, 0.33 mmol/L 4-aminoantipyrine, 4 mmol/L phenol, 2 g/L non-ionic surfactant, and commercial biocides.
R2: 5.18 mmol/L cholesterol standard.

Procedure: To the labeled test tubes, 1.0 ml of the working reagent (R1) was mixed with 10 µl of serum sample or 10 µl of standard cholesterol solution. After 5 minutes of incubation at room temperature, the absorbance was measured at 546 nm against the reagent blank.

Serum Total Cholesterol Concentration Calculation: Serum total cholesterol concentration is calculated as follows:

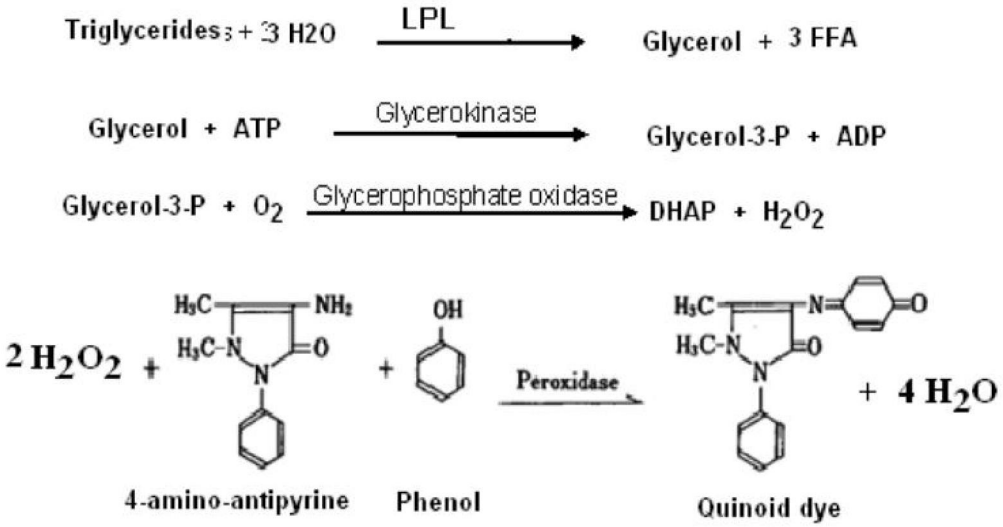
Total Cholesterol (mg/dL) = (A sample / A standard) X Concentration of standard

Where A is the absorbance at 546 nm of solutions after reactions were completed.

3.12.4 Determination of Serum Triglycerides (TGAs)

Serum triglycerides were estimated by using a commercial kit, based on the method developed by obtained from Cromatest® Cholesterol MR, Linear chemicals SL, Barcelona, Spain [Allain *et al.,* 1974].

Principle: The method is based on the initial enzymatic hydrolysis of triglycerides to glycerol and free fatty acids (FFA) by lipoprotein lipase (LPL), followed by three further enzyme steps that eventually produce a colored product that is quantitatively proportional to the concentration of triglyceride in the serum sample being tested. The glycerol generated is phosphorylated by glycerol kinase (GK) to glycerol-3-phosphate, which is then oxidized by glycerol phosphate oxidase (GPO) to form di-hydroxyacetone phosphate (DHAP) and hydrogen peroxide (H2O2). With peroxidase (POD) and H2O2, the chromogenic substrate, 4-aminoantipyrine, is coupled with phenol to form a colored product, whose optical density is measured at 500 nm.



Reagents: R1: 150000 U/l lipoprotein lipase, 800 U/l glycerol kinase, 4000 U/l glycerol-3- P-oxidase, 440 U/l Peroxidase, 0.7 mmol/l 4-Aminoantipyrine, 0.3 mmol/l ATP and 7.5 mmol/l phenol. R2: Glycerol equivalent to a concentration of 200 mg/dl (2.28 mmol/l) triglycerides.

Procedure*:* To labeled test tubes, 1.0 ml of reagent R1 was mixed with 10 µl of patient serum sample or 10 µl of glycerol standard (R2). After 5 minutes of incubation at room temperature, the absorbance was measured at 500 nm against the reagent blank.

Calculation: Triglyceride concentration in the samples was calculated against the absorbance of the standard as follows:

Triglycerides (mg/dL) = (A sample / A standard) X Concentration of the standard

Where A=absorbance at 500 nm.

3.12.5 Serum HDL-cholesterol Level

HDL-cholesterol in the patient‘s blood samples was estimated by using a commercial kit, based on the fact that Apo lipoproteins B-containing lipids, namely chylomicrons, LDL and VLDL can be selectively precipitated from serum using phosphotungstic acid, leaving HDL in the supernatant obtained from Cromatest ®-Cholesterol MR, Linear Chemicals SL, Barcelona, Spain [Wahlefeld, 1974].

Principle: Chylomicrons, VLDL and LDL are precipitated with phosphotungstic acid and magnesium chloride and removed by centrifugation. The supernatant contains HDL only as a significant source of cholesterol, and was used for estimating cholesterol by using a kit as mentioned above for total cholesterol determination.

Reagents and Procedure: Precipitating reagent: made from four parts by volume of 0.55 mmol/L phosphotungstic acids, 25 mmol/L MgCl2, plus one part by volume of deionizer water.

Assay Reagent: 200 mmol/L PIPES pH 7.0, containing 1 mmol/L sodium cholate, > 250 U/L cholesterol esterase, > 250 U/L cholesterol oxidase, >1 KU/L peroxidase, 0.33 mmol/L 4-aminoantipyrine, 4 mmol/L phenol and 2 g/L non-ionic surfactant, plus unspecified biocides.

First, the precipitating reagent (500µL) was mixed with 200 µL of serum and incubated for 10 minutes at room temperature. The incubated mixture was centrifuged for 10 minutes at 4000 g and the clear supernatant, containing the HDL, was separated from the precipitate.

To labeled test tubes, 1.0 ml of the Assay Reagent was mixed with 10 µl of clear supernatant containing the HDL. After 5 minutes of incubation at room temperature, the absorbance of the sample and standard HDL solutions were measured at 546 nm against the reagent blank.

3.12.6 Serum LDL-cholesterol Level

A method developed by Friedwald‘s is used to estimate LDL-C level. The method involves measurements of fasting total cholesterol, triglyceride and high-density lipoprotein. Total cholesterol concentrations and calculating the value of LDL-c by using Friedwald‘s formula. In Friedwald‘s formula, TG replaces VLDL, because it has been shown that serum TG levels are equivalent to five times the levels of VLDL. Friedwald‘s formula:-

LDL-C = Total Cholesterol – [HDL-C + (TG/5)].

3.12.7 Determination of Serum Creatinine

Serum creatinine was measured using the modified Jaffe method. In alkaline solution, Creatinine forms a yellow orange complex with picrate and this colour intensity is directly proportional to the creatinine concentration. This was measured spectrophotometrically at 510nm [Peake & Whiting, 2006].

Procedure: Working reagent, sample and standard were pre-incubated at 37 °C. The spectrophotometer was adjusted to zero absorbance with air. Working reagent (10µl) and sample (10µl) or standard (10µl) were pipetted into cuvette and mixed gently. The cuvettes were put into the cell holder and stopwatch started to count. The absorbance was recorded at 400 nm after 30 seconds (A1) and after 90 seconds (A2) of the sample or standard addition.

3.12.8 Determination of Serum Urea

Urea was measured by the decrease in NADH absorbance per unit time. Urea is hydrolyzed by urease to produce carbon dioxide and ammonia. The produced ammonia combines with 2-oxogluterate and NADH yielding glutamate and NAD^+. The reaction is monitored kinetically at 340 nm by the rate of the decrease in the absorbance resulting from the oxidation of NADH to NAD^+, proportional to the concentration of urea present in the sample [Fawcett&Scott,1960]

4. RESULTS

This study was a hospital based, cross sectional study to compare blood glucose level, lipid profile and renal function values between type-2diabetic patients who received non-insulin based therapies, and those who received DPP-4 inhibitors based therapies in the Diabetic Clinic of Tikur Anbessa Specialized Hospital and Huleshet Higher Private Clinic respectively.

4.1 Demographic characteristics of diabetic patients

A total of 90 patients diagnosed withT2DM. Sixty type-2 diabetic patients who received non-insulin based therapies and 30 type-2 diabetic patients who received DPP-4 inhibitors based therapies were selected in this study. Among a total of 90 type-2 diabetic patients diagnosed, 47(52.2%) were Females and 43 (47.8%) were Males. Out of the 60 diabetic patients who received non-insulin based therapies 37(61.7%) were Females and 23(38.3%) were Males and out of the 30 diabetic patients who received DPP-4 inhibitors based therapies 10 (33.3%) were Females and 20(66.7%) were Males.

Among diabetic patients who received non-insulin based therapies 4 (6.7%) patients were found to take alcohol and 56 (93.3%) patients did not. Among diabetic patients who received DPP-4 inhibitors based therapies 8 (27.7%) patients confirmed they drink alcohol and 22 (73.3%) were non drinkers. Moreover, the patients in the non-insulin based group are non-smokers but from patients receiving DPP-4 inhibitors based therapies 5 (27.7%) are smokers and 25 (73.3%) are non-smokers.

Among diabetic patients who received non-insulin based therapies 10 (17.7%) had family history of diabetes and 50 (83.3%) patients had no family history. Among diabetic patients who received DPP-4 inhibitors based therapies 5 (17.7%) patients had family history of diabetes and 25 (83.3%) patients had no family history. BMI of the study participants was also calculated using their weight and height. The minimum value was 15.80 kg/m^2 and the maximum was 40.80 kg/m^2. Among the non-insulin based treated patients, 21 (35.00%) had normal body mass index, 23 (38.30%) were found to be overweight, 13 (21.70%) were found to be obese and 3

(5.00%) were underweight. However, in the DPP-4 inhibitors based treated diabetic patients group 7 (23.30%) were normal 1(3.30%) was overweight, 22 (73.30%) were obese and none of the study participants were found to be underweight as shown in table 1.

Table 1: Socio- demographic characteristics of diabetic patients in the study

Variable	Non-insulin based treated diabetic Patients (N=60)		Incretin-based treated diabetic Patients (N=30)	
	Number (N)	Percentage (%)	Number (N)	Percentage (%)
Sex				
Female	37	61.7%	10	33.3%
Male	23	38.3%	20	66.7%
Age in years				
18-30	-	-	-	-
31-40	5	8.3%	1	3.3%
41-50	10	16.7%	9	30.0%
51-60	24	40.0%	10	33.3%
>60	21	35.0%	10	33.3%
Duration of diabetes in years				
<5	1	1.7%	2	6.7%
5-10	20	33.3%	20	66.7%
>10	39	65.0%	8	26.7%
Family History of diabetes				
Yes	10	17.7%	5	17.7%
NO	50	83.3%	25	83.3%
Smoking status				
Yes	0	-	5	17.7%
No	60	100%	25	83.3%
Alcohol consumption				
Yes	4	6.7%	8	27.7%
No	56	93.3%	22	73.3%
BMI in kg/m^2				
<18.5	3	5.0%	-	-
18.5-24.9	21	35.0%	7	23.3%
25-29.9	23	38.3%	1	3.3%
>30	13	21.7%	22	73.3%

The average age of diabetic patients who received non-insulin based therapies was 56.48 ± 9.75 years, ranging between 35 and 78 years. The average age of diabetic patients who received DPP-4 inhibitors based therapies was 56.50 ± 9.77 years, ranging between 32 and 72 years. The mean duration of diabetes who received non-insulin based therapies was 14.92 ± 6.25 years, ranging from 3 to 31 years. The mean duration of diabetes who received DPP-4 inhibitors based therapies was 9.627 ± 4.56 years, ranging from 3 to 22 years. The mean values of lipid profile, fasting blood sugar, renal function, and body mass index of all diabetic patients from base line is shown in table 2. The study finding shows that there were no significant differences in the mean values of fasting blood sugar, body mass index, lipid profile and renal function test except TC and duration of diabetes as shown in table 2.

Table 2:- The baseline characteristics of all diabetic patients

Biochemical Parameters	**DPP-4 inhibitors based treated diabetic patients**	**Non-insulin based treated diabetic patients**	**P-value**
Sex(female/male)	10/20	37/23	-
Age (years)	56.5±9.7	56.1±10.1	0.847
Duration of diabetes (years)	9.62±4.5	14.92±6.25	0.000*
FBS (mg/dl)	188.08±78.743	171.73±72.720	0.331
TC (mg/dl)	171.87±49.821	196.65±40.385	0.013*
TG (mg/dl)	184.23±53.742	185.60±71.603	0.927
LDL (mg/dl)	115.33±37.921	125.88±39.398	0.227
HDL (mg/dl)	35.83±13.143	36.12±7.925	0.899
urea (mg/dl)	26.53±8.274	26.50±8.345	0.986
Creatinine (mg/dl)	0.94±0.117	0.956±0.118	0.826
BMI(kg/m^2)	25.35±3.05	25.04±3.98	0.800

***The mean is significant at the p< 0.05 (By using independent t test analysis)**

The percentages of patients taking different anti-diabetic drugs were different within the study group. Eleven types of anti-diabetes Drugs were used in the clinic during the study period and they were used either as single therapy or combinations. Majority of the diabetic patients who received non-insulin based therapies were treated on metformin with Neutral Protamine Hagedorn (NPH) 29 (32.2%) as combination therapy and majority of the patients who received DPP-4 inhibitors based therapy were treated on Galvusmet 11(12.2%). Majority of the diabetic patients who received non-insulin based therapies were treated on metformin 14 (15.6%) and majority of the patients who received DPP-4 inhibitors based therapy were treated on Onglyza 7(7.8%) between the groups on single therapy.

The doses of the drugs were metformin (500-2000mg/day), glibenclamide (2.5-20mg/day), insulin with various concentrations. Glibenclamide and insulin were taken BID 20-30 min before food while metformin 30 min after food for individuals who received non-insulin based therapies. The concentration of the drugs given to patients in DPP-4 inhibitors based therapy group were Sitagliptin (Januvia) with 25mg, 50mg, 100mg tablets and also in combination with metformin (Janumet) with 50/500mg, Vildagliptin (Galvus) with 50mg tablets and also in combination with metformin (Galvusmet) with 50/500mg, Saxagliptin (Onglyza) with 5mg tablets and also in combination with metformin (Konbolyza) with 5/500mg as shown in table 3.

Table 3: Percentage of Anti-diabetic Medications prescribed to patients during the study period.

Type of therapies	Drugs	Frequency	Percent
Non-insulin based treated diabetic patients	Metformin	14	15.6
	Metformin+NPH	29	32.2
	Glibencamide+Metformin	4	4.4
	Glibencamide	7	7.8
	Glibencamide +NPH	6	6.7
DPP-4 inhibitors based treated diabetic patients	Januvia	4	4.4
	Janumet	2	2.2
	Galvus	5	5.6
	Galvusmet	11	12.2
	Onglyza	7	7.8
	Konbolyza	1	1.1
	Total	90	100.0

4.2. Clinical characteristics of diabetic patients

4.2.1 Blood Glucose Level

The mean FBS level of diabetic patients who received DPP-4 inhibitors based therapies was found to be 152.87±51.977 mg/dl and the mean FBS level of diabetic patients who received non-insulin based therapies was 189.34±81.520 mg/dl. Taking 126 mg/dl as a reference point, greater proportion of the patients 66 (73.4%) were found to be above the reference point. The mean FBS level of diabetic patients who received DPP-4 inhibitors based therapies was statistically significant (p=0.028) lower than that of diabetic patients who received non-insulin based therapies as shown in table 4. Among diabetic patients, females had higher level of FBS as compared to males. This difference between the two groups was not statistically significant (p>0.05) as shown in table 7.

4.2.2 Lipid Profile: TC, TG, LDL and HDL Concentrations

All serum lipid profiles were significantly lower in diabetic patients who received DPP-4 inhibitors based therapies as compared to diabetic patients who received non-insulin based therapies. The mean level value of lipid profiles (TC, TG, and LDL) in diabetic patients who received DPP-4 inhibitors based therapies was significantly lower than the mean serum of diabetic patients who received non-insulin based therapies (p=0.028, P=0.000,and P=0.002) respectively and higher mean level of HDL (p=0.003) as shown in table 4. Among diabetic patients, female had higher level of TC, TG, LDL and low HDL as compared to male. This difference was statistically not significant (p>0.05) as shown in table 7.

4.2.3 Renal function test: serum creatinine and serum urea Concentrations

The mean value of serum creatinine level of diabetic patients who received DPP-4 inhibitors based therapies was significantly (p=006) lower as compared to that of diabetic patients who received non-insulin based therapies. The mean serum urea value of diabetic patients who received DPP-4 inhibitors based therapies was significantly (p=000) lower as compared to that of diabetic patients who received non-insulin based therapies as shown in table 4.

4.2.4 Body mass index

BMI of the study group was also calculated using their weight and height. Based on the calculation, the mean BMI of diabetic patients who received DPP-4 inhibitors based therapies were lower as compared to that of diabetic patients who received non-insulin based therapies. This differences were statistically not significant ($p>0.05$) as shown in table 4.

Table 4: Comparison of Mean ± SD of serum lipid profile (TC, TG, LDL, and HDL), renal function test (serum urea, serum creatinine), body mass index(BMI) and fasting blood sugar level (FBS) of all diabetics with non-insulin based therapies and DPP-4 inhibitors based treated diabetic patients.

Biochemical Parameters	DPP-4 inhibitors based treated diabetic patients (n=30)	Non-insulin based treated diabetic patients (n=60)	P-value
FBS (mg/dl)	152.87±51.977	189.34±81.520	0.028*
TC (mg/dl)	142.47±41.755	188.25±50.501	0.000*
TG (mg/dl)	147.70±74.026	160.50±90.264	0.504
LDL (mg/dl)	88.47±31.837	116.33±42.698	0.002*
HDL (mg/dl)	44.77±10.434	36.67±12.361	0.003*
urea (mg/dl)	17.20±4.567	34.03±9.154	0.000*
creatinine(mg/dl)	0.707±0.326	0.989±0.181	0.006*
BMI(kg/m^2)	24.1±3.63	26.6±4.21	0.108

Mean ± SD is significant at the $p< 0.05$ (by Independent Samples Test)

Remarks: FBS =fasting blood glucose, TC= total cholesterol, HDL = high density lipoprotein, LDL = low density lipoprotein, BMI=body mass index.

The mean FBS values of DPP-4 inhibitors based treated diabetic patients was found to be reduced by 35.21 ± 26.77 mg/dl and the mean BMI value was also reduced by1.25kg ±0.58 from baseline. This difference was not statistically significant (p>0.05). The mean lipid profile (TC, TG, LDL and HDL) and renal function test (serum urea and serum creatinine) values of DPP-4 inhibitors based treated diabetic patients showed a statistically significant reduction from baseline (p<0.05) as shown in table 5.

Table 5: Comparison of Mean ± SD of serum lipid profile, renal function test, FBS and BMI of all diabetics with DPP-4 inhibitors based treated diabetic patients from baseline.

Biochemical Parameters	DPP-4 inhibitors based treated diabetic patients		P-value
	Baseline	Final	
FBS (mg/dl)	188.08±78.743	152.87±51.977	0.050
TC (mg/dl)	171.87±49.821	142.47±41.755	0.015*
TG (mg/dl)	184.23±53.742	147.70±74.026	0.021*
LDL (mg/dl)	115.33±37.921	88.47±31.837	0.006*
HDL (mg/dl)	35.83±13.143	44.77±10.434	0.015*
urea (mg/dl)	26.53±8.274	17.20±4.567	0.000*
Creatinine (mg/dl)	0.94±0.117	0.707±0.326	0.012*
BMI(kg/m^2)	25.35±3.05	24.1±3.63	0.232

***Mean ± SD is significant at the *p*< 0.05 (by Paired t- test)**

The mean FBS values in case of non-insulin based treated diabetic patients was found to be increased by 17.61 ± 8.8 mg/dl and the mean BMI value also increased by1.56kg ±0.23 from baseline but statistically not significant (p>0.05). The mean lipid profile (TC, TG and LDL) values were reduced from baseline and mean value level of HDL increased from baseline but statistically not significant (p>0.05).The mean serum urea value level were significantly increased (p<0.05) from base line and the mean value serum creatinine level were increased but statistically not significant (p>0.05) from base line as shown in table 6.

Table 6: Comparison of Mean ± SD of serum lipid profile, renal function test, FBS and BMI of all diabetics with non-insulin based treated diabetic patients from base line.

Biochemical Parameters	Non-insulin based treated diabetic patients		P-value
	Baseline	Final	
FBS (mg/dl)	171.73±72.720	189.34±81.520	0.236
TC (mg/dl)	196.65±40.385	188.25±50.501	0.352
TG (mg/dl)	185.60±71.603	160.50±90.264	0.111
LDL (mg/dl)	125.88±39.398	116.33±42.698	0.244
HDL (mg/dl)	36.12±7.925	36.67±12.361	0.787
urea (mg/dl)	26.50±8.345	34.03±9.154	0.000*
Creatinine (mg/dl)	0.956±0.118	0.989±0.181	0.732
BMI(kg/m^2)	25.04±3.98	26.6±4.21	0.502

Mean ± SD is significant at the $p< 0.05$ (by Paired t- test)

The mean values of FBS, BMI, TC, TG, LDL, and Serum urea were increased in the female as compared to male type-2 diabetic patients in both groups and low HDL and serum creatinine as compared to male. However, the mean difference of females diabetic patient of FBS, lipid profiles and serum urea were statistically not significant ($p>0.05$) as compared to males diabetic patients and there were significant difference in the mean value level of serum creatinine and BMI of females diabetic patients as compared to males diabetic patients ($p<0.05$) as shown in table 7.

Table 7: Comparison of Mean ± SD of serum lipid profile, renal function test (serum urea, serum creatinine), FBS and BMI of all diabetic patients with DPP-4 inhibitors based treated diabetic patients and non-insulin based treated diabetic patients in relation to gender.

Biochemical parameters	Sex of study participants	N	Mean	Std. Deviation	p-value
FBS in mg/dl	Female	47	187.71	69.366	0.164
	Male	43	165.67	79.402	
TC in mg/dl	Female	47	177.53	52.345	0.391
	Male	43	168.02	52.300	
TG in mg/dl	Female	47	166.79	86.843	0.220
	Male	43	144.70	82.378	
LDL in mg/dl	Female	47	110.72	40.539	0.381
	Male	43	103.02	42.415	
HDL in mg/dl	Female	47	38.98	12.229	0.757
	Male	43	39.79	12.527	
Serum urea in mg/dl	Female	47	29.21	10.691	0.488
	Male	43	27.56	11.849	
Serum creatinine in mg/dl	Female	47	0.651	0.2781	0.000*
	Male	43	0.893	0.2667	
BMI in kg/m^2	Female	47	25.938	4.3413	0.030*
	Male	43	24.156	3.1964	

Mean ± SD is significant at the $p < 0.05$ (by Independent Samples Test)

Variables (factors) like age, BMI and duration of diabetes which may affect lipid profiles, renal function tests, and FBS level were correlated. BMI correlated positively with the levels of TC (r=0.154), TG (r=0.162), LDL-C (r=0.077) and negatively with HDL-C (r=0.148) but the correlation was statistically not significant (p>0.05) as shown in figure 5.The BMI value was also correlated positively with serum urea level (r=0.023), FBS level (r=0.099) and correlated negatively with serum creatinine level (r=0.099) but statistically not significant (p>0.05) as shown in figure 6.

Further correlation studies were conducted between FBS, TC, TG, LDL-C, HDL-C, serum urea and serum creatinine level with duration of diabetes. A positive correlation was obtained with the level of TC (r=0.247), TG (r=0.139), LDL-C (r=0.251), serum urea (r=0.369),FBS (r=0.219) and negative correlation with the level of HDL-C (r=0.056) and serum creatinine (r=0.167) But, there was significant moderate positive correlation between TC,LDL-C, serum urea and FBS (p<0.05) as shown in figure(7,8, 9&10) respectively.

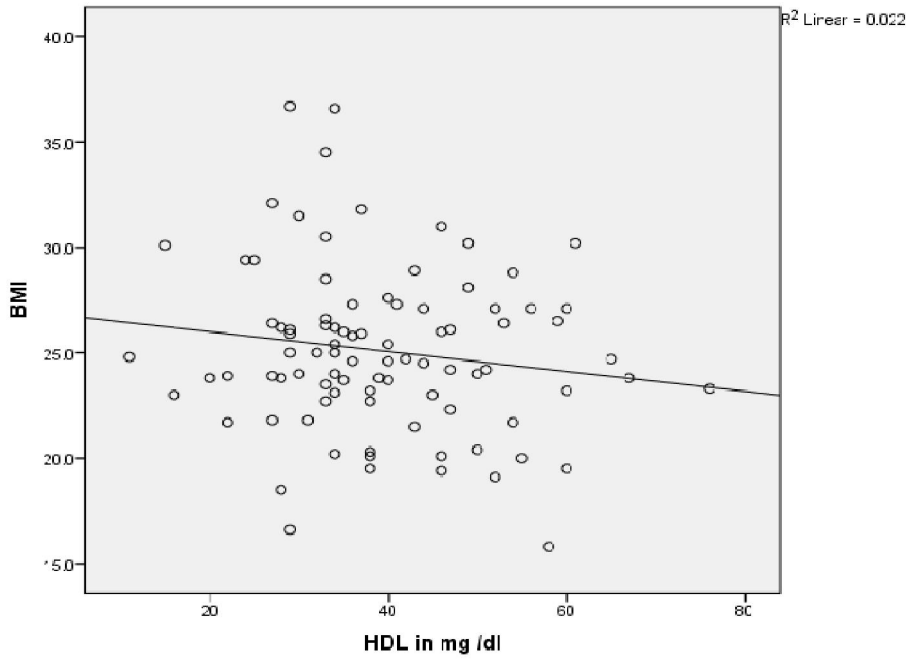


Figure- 5: Correlation between BMI and HDL-C concentrations

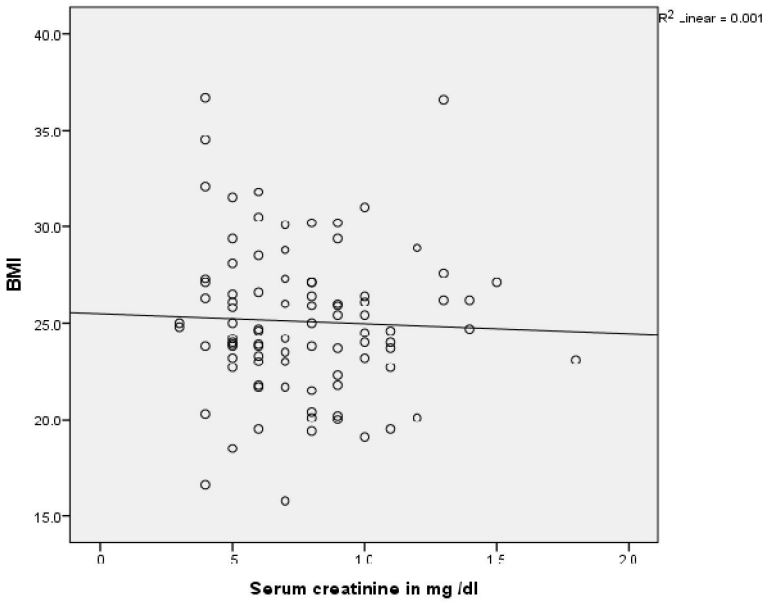


Figure-6: Correlation between BMI and Serum creatinine concentrations

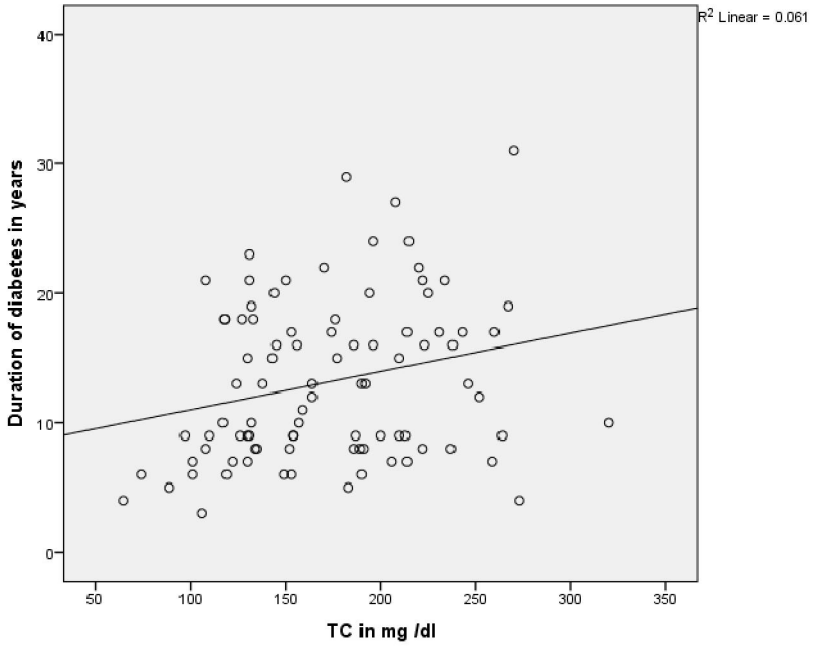


Figure-7: Correlation Between duration of diabetes mellitus and TC concentrations

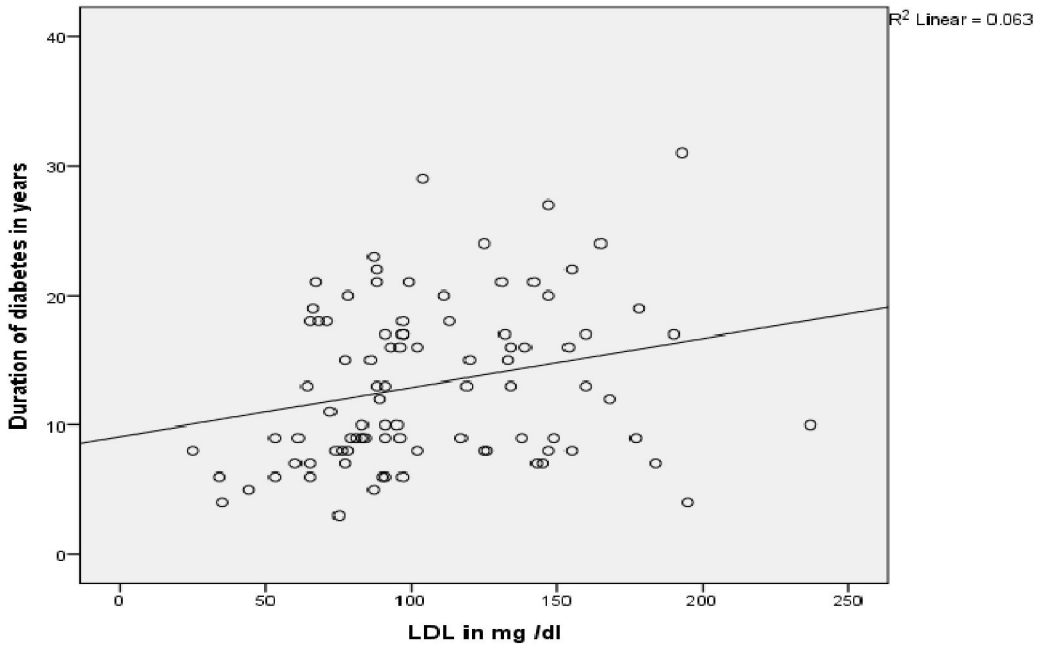


Figure-8: Correlation between duration of diabetes mellitus and LDL-C concentrations

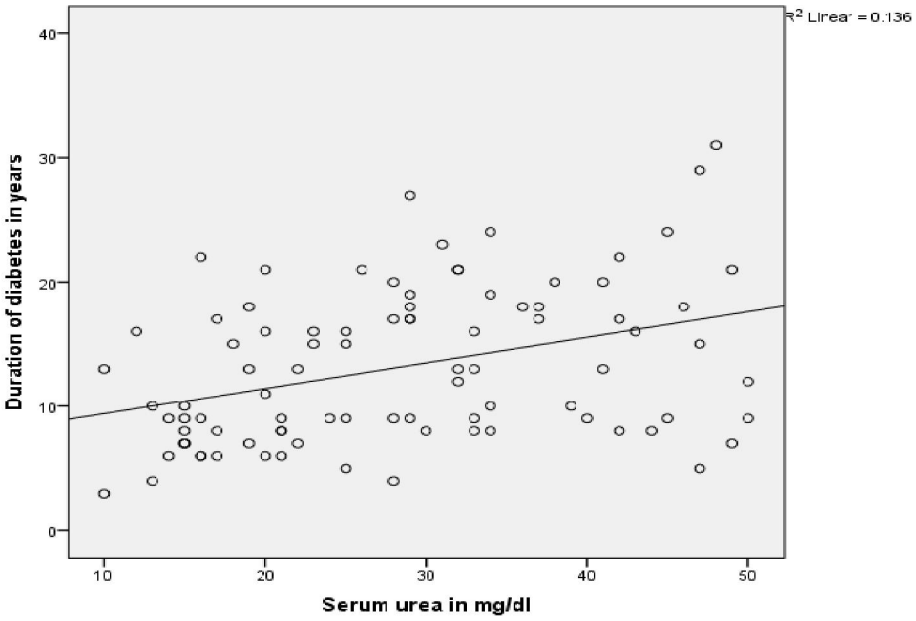


Figure-9: Correlation between duration of diabetes mellitus and Serum urea concentrations

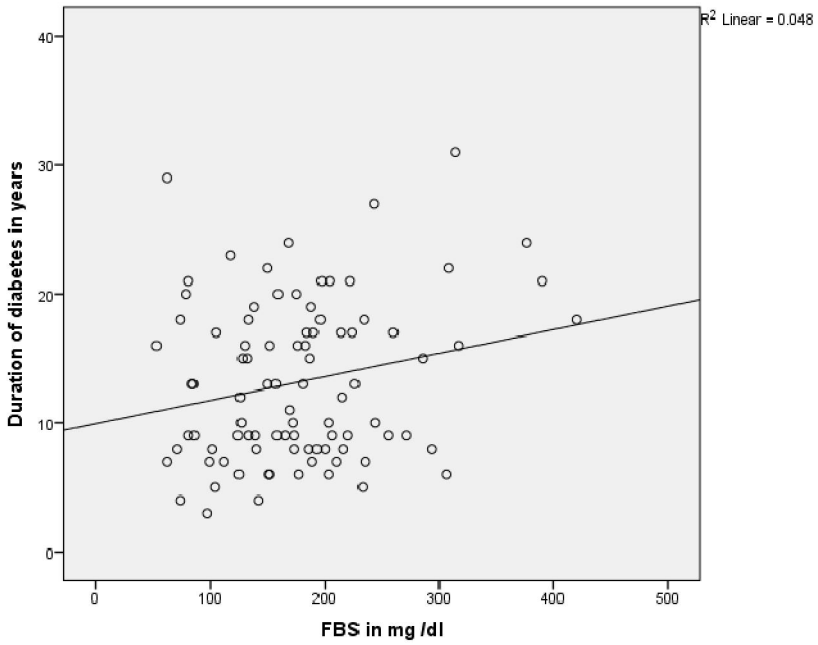


Figure-10: Correlation between duration of diabetes mellitus and FBS concentrations

5. DISCUSSION

Diabetes mellitus is a serious chronic disease that is growing rapidly, affecting nearly 382 million people worldwide, and requires continuous medical care once diagnosed. The epidemic of T2DM and the recognition that achieving specific glycemic goals can substantially reduce morbidity have made the effective treatment of hyperglycemia a top priority [ADA, 2012]. Because current treatment regimens for T2DM do not effectively target the fundamental defects in glucose-mediated insulin secretion and beta-cell loss, an increasing proportion of T2DM patients progress to requiring insulin.

The majority of OHAs in common use are insulin sensitizers and/or insulin secretagogues. Older patients, in particular, are vulnerable to impaired awareness of hypoglycemia with consequent neuroglycopenia and adverse cardiovascular effects, dictating the need for particular caution with therapies that increase the risk of hypoglycemia. Significantly, in the ACCORD trial, combination therapy using high doses of thiazolidinediones (TZD), sulfonylurea (SU), metformin, and insulin, was associated with an increase in cardiovascular and all-cause mortality, possibly because of hypoglycemia. Metformin and TZDs decrease insulin resistance and hepatic glucose output, but are contraindicated in patients with significant renal and/or cardiac dysfunction, both of which occur frequently in T2DM [ACCORD, 2008].

Accordingly, the recent advent of so-called 'incretin-based therapies', the incretin hormones being glucagon like peptide-1 (GLP-1) and glucose dependent insulinotropic peptide (GIP), which have the potential to address these defects, represents a major paradigm shift in management of T2DM patients. The actions of incretins have effect on the defects in glucose metabolism, pancreatic function and energy production in T2DM patients. These findings have led to the development of once-daily, orally-active DPP-4 inhibitors to increase the incretin effect. DPP-4 activity is reduced by almost 100% within 15 to 30 minutes of oral administration of the DPP-4 inhibitors sitagliptin or vildagliptin, producing a 2-fold increase in mean active GLP-1 levels, with a duration of inhibition in excess of 16 hours because of initial rapid binding to DPP-4 inhibitors, followed by a slow phase of tight binding, so that effects persist for 24 hours after administration of a single dose of sitagliptin [Khoo *et al*., 2009].

DPP-4 inhibition increases GLP-1 and GIP levels by 2- to 3-fold, while reducing glucagon, although the magnitude of the rise in GLP-1 is dependent on the type of nutrient ingested. DPP -IV inhibitors have good tolerability and are associated with very few side effects. Both vildagliptin and sitagliptin are safe and well-tolerated as monotherapy or as combination therapy. No drug-related serious adverse events were reported after 12 weeks of vildagliptin and sitagliptin as monotherapy or in combination with metformin. During the study it has been found that T2DM affected both the sex equally and mostly it is pronounced at the age of 56.5±9.7 years (Kapadia *et al*., 2014).

To our knowledge, no clinical studies have been specifically made in Ethiopia, to show the relationship between DPP-4 inhibitors based therapies and the non-insulin based therapies among T2DM on lipid profile and renal function test values since the drugs were recently introduced in Ethiopia as a treatment strategy. Therefore, this study was done to unravel which of the strategies better reverse glycemic conditions and also improve lipid profiles in order to avoid diabetic complications. The study also aimed to compare the effect on glycemic control, weight gain, and frequency of hypoglycemia in patients receiving DPP-4 inhibitors based therapies or non-insulin based therapies among T2DM.

Our results regarding plasma glucose indicate that there was an improvement after treatment courses of six month with DPP-4 inhibitors based therapies' either as mono therapy or combination with metformin as compared to that of diabetic patients who have received non-insulin based therapies either as monotherapy or in combination therapy.

The FBS level of diabetic patients who were treated with DPP-4 inhibitors based therapies was lower than that of diabetic patients who were treated with non-insulin based therapies and the reversal effect was very significant. This could be because such treatments may stimulate insulin secretion, reduce gastric emptying thereby improving appetite and also inhibit glucagon secretion. Karen and his group obtained the same type of result [Karen *et al*., 2010]. However, despite the fact that these patients were having follow-ups in the two diabetic clinics, their blood sugar level was still in the hyperglycemic range showing they had poor glycemic control. The reason for this could be multifaceted: one reason could be that patients do not adhere to

their regiments or they don't observe the exact time of taking their drugs; or it could be that they did not have a properly scheduled follow up to scale up their doses. Recently, fixed combinations of sitagliptin and vildagliptin with metformin have also been launched; these affect pathogenic factors of T2DM at more target points: they reduce the extent of insulin resistance, regulate insulin secretion in a glucose-dependent way, reduce glucagon secretion and also decrease hepatic glucose production [Nauck, Vilsboll *et al*., 2009].

The promising therapeutic potential of DPP-4 inhibitors based therapies as a pharmacological tool for treating T2DM has been discovered in the 1990s, the gliptins increase insulin production and release as well as reduce glucagon levels in a glucose-dependent way, resulting in a decrease of fasting and postprandial glycemia, as well as HbA1c levels as compare sulfonylureas, metformin and there combination [Nauck, Vilsboll *et al*. 2009]. These results demonstrate that treatment with incretin-based therapies was more efficacious than patients who have received non-insulin based therapies via improving glycaemia. Therefore, there is considerable interest in enhancing incretin action for treatment of T2DM as a plausible and promising therapeutic strategy for control of blood glucose level.

As presented in table 4, mean values of BMI were lower in DPP-4 inhibitors based treated diabetic patients than those treated with non-insulin based treated patients but the difference was not statistically significant ($p>0.05$). This implies that the DPP-4 inhibitors based therapy improves the body mass index. Diabetic patients receiving non-insulin based treatment showed weight gain which contributes to the increase in BMI. Similar results were reported by other researchers [Monami *et al*., 2010] in which the patients on DPP-4 inhibitors based therapy appeared to have slightly lower BMI than non-insulin based treated T2DM.

The body weight normalizing effect of DPP-4 inhibitors may prevail through several mechanisms which include the following [Foley & Jordan, 2010]: After a meal that is rich in fat, DPP-4 inhibitor treatment reduces the level of chylomicron apoB-48 and so it hinders intestinal triglyceride absorption and Postprandial catecholamine (nor-epinephrine) levels increase upon the administration of DPP-4 inhibitors, resulting in an increased lipolysis in the adipose tissue and fatty acid oxidation in the musculature.

Other studies showed , that DPP-4 inhibitors prolongs and enhances the activity of endogenous GLP-1 and GIP, which serves as important Postprandial stimulators of insulin secretion and regulators of blood glucose control. In clinical trials DPP-4 inhibitors have shown efficacy and tolerability in management of hyperglycemia in the type 2 diabetes, without causing weight gain or hypoglycemia [Ducker &Nauck, 2006].

Findings of our study indicated that DPP-4 inhibitors based therapy also improves the lipid profiles of type 2 diabetic patients better than the patients that were under non-insulin based treatment. When the data for lipid profiles of DPP-4 inhibitors based treated diabetic patients were compared with that of non-insulin based treated diabetic patients the former treatment strategies showed a better adjustment of lipid profiles to baseline values. The mean values of TC, TG, LDL-C of DPP-4 inhibitors based treated diabetic patients was significantly lower than non-insulin based treated diabetic patients as shown in our result in table 4. However, the HDL-C was increased in the DPP-4 inhibitors based treated diabetic patients than the non-insulin based treated diabetic patients. The present study showed the mean values of lipid profile of diabetic patients who received DPP-4 inhibitors based therapies were significantly lower than the mean values of diabetic patients from the baseline as shown in our result in table 5.

On the other hand, the mean values of lipid profile of diabetic patients who received non-insulin based therapies patients lower than from baseline but statistically not significantly ($p > 0.05$) as shown in table 6. This supports the idea that DPP-4 inhibitors based treatment strategy better improves lipid profiles and hence curbs the possibility or chance of diabetic patients to develop dyslipidemia that could lead to different types of diabetic complications like hypertension, atherosclerosis and heart attack.

Lipid profile plays an important role in cardiovascular risk assessment. One of the major risk factors for the development of cardiovascular disease is dyslipidemia, which may be primary or associated with hypertension, diabetes mellitus and obesity. T2DM and dyslipidemia are important risk factors for cardiovascular disease. Elevated serum levels of LDL-C and TG and low levels of HDL-C are strongly associated with increased risk for macrovascular events (e.g., myocardial infarction, ischemic stroke, and coronary heart disease) among patients with T2DM [Mohieldein *et al., 2014]*. Animal studies suggested that incretin hormones reduce intestinal TG absorption and increased chylomicron metabolism [Wasada *et al*., 1989].

The present study showed the mean values of renal function test as shown in tables 7, the mean level value of serum urea of diabetic patients who received based therapies was significantly ($p<0.05$) lower than the mean value of diabetic patients who received non-insulin based therapies subjects. The mean values of serum creatinine were also lower than that of diabetic patients who received non-insulin based treated diabetic patients but statistically was not significantly ($p> 0.05$) as shown in table 7. The mean value level of serum urea diabetic patients who received DPP-4 inhibitors based therapies were significantly lower ($P<0.05$) as compared to base line as shown in table 5. Similar studies showed that sitagliptin may be used as monotherapy in patients who cannot tolerate metformin or sulfonylurea, and sitagliptin may be used as alternative to metformin in renal insufficiency [Gonzalis *et al*., 2008].

6. CONCLUSIONS

Diabetes is a non-communicable disease which was not considered as a health threat in Ethiopia. However, due to change in dietary habits and life style the scenario has completely changed. This present work shows that DPP-4 inhibitors based therapy for treatment of T2DM either as monotherapy or in combination therapy corrects lipid profile, renal function values, fasting blood sugar and weight gain as compared to non-insulin based therapies either as monotherapy or in combination for treatment of T2DM. Generally, DPP-4 inhibitors based therapy showed clinically significant improvement in lipid profile correction and renal function. DPP-4 inhibitors based therapy administration is particularly beneficial in overweight and obese patients who represent the majority of patients with T2DM, as well as in elderly patients and in diabetics who are susceptible to hypoglycemia. The advantage of the DPP-4 inhibitors based therapeutic strategies is that, since they in a better way adjust lipid profiles and blood sugar levels of patients, eventually they also presumably curb the through time development of diabetic complications like hypertension, nerve ending problems, retinopathy, cardiovascular diseases, obesity, and renal problems.

7. LIMITATIONS OF THE STUDY

The limitations in the present study include: The number of patients was small to make a reliable conclusion. We wanted to make assessment of HbA1c for the patients but the cost was as high as 100ETB per sample, which was way beyond the fund given in total for the project. Such determination would have given a better picture of glycemic control of patients. Also, this short cross- sectional study could not follow up the patients, who were taking anti-diabetic drugs for long duration of biochemical and enzymatic progression due to limitation of money and time. They did not include control group of diabetic subjects due to limitation of money.

8. RECOMMENDATIONS

The recommendations in the present study include: We recommend to introduce DPP-4 inhibitors based therapy as a better therapeutic strategy in the treatment of diabetes T2DM. Though all the patients had follow-up, most are still hyperglycemic and this is not encouraging. This may be because patients get usually 6 months appointments with their doctors. This implies that patients do not have a proper clinical follow-up. Hence we recommend that patients must get their doctors for consultation at least every 3 months. We also strongly recommend for a proper control of substandard drugs coming to the country by the concerned authorities. Only drugs with high analytical grade purity and efficacy need to be allowed for importation. A proper awareness and health education needs to be given to patients and the whole public regarding the disease its medication and precautions to be taken by the public. Non-communicable diseases shall be given due attention and the Ministry of health shall design a strategy to curb the issue.

REFERENCES

Action to Control Cardiovascular Risk in Diabetes Study Group (**2008**). Effects of intensive glucose lowering in type 2 diabetes. *The New England journal of medicine*, *358*(24), p.2545.

Ahrén, B. (**2013**). Incretin therapy for type 2 diabetes: GLP-1 receptor agonists and DPP-4 inhibitors. *European Diabetes Nursing*, *10*(1), p.31-36.

Allain, CC., Poon, LS., Chan, CS., Richmond, WF. and Fu, PC. (**1974**). Enzymatic determination of total serum cholesterol. *Clinical chemistry*, *20*(4), p.470-475.

American Diabetes Association (**2001**). *Diabetes is a Silent Killer*.

American Diabetes Association (**2003a**). Standards of medical care for patients with diabetes mellitus. Position statement. Diabetes Care, 26, p.33–50.

American Diabetes Association (**2003b).** Evidence-based nutrition principles and recommendations for the treatment and prevention of diabetes and related complications. Position statement. Diabetes Care, 26, p. 51–61.

American Diabetes Association (**2012).** Diagnosis and classification of diabetes mellitus.Diabetes.

Baggio, LL. and Drucker, DJ. (**2007**). Biology of incretins: GLP-1 and GIP. *Gastroenterology*, *132*(6), p.2131-2157.

Capriotti, T. (**2005**). Type 2 diabetes epidemic increases use of oral anti-diabetic agents. *Medsurg Nursing*, *14*(5), p.341.

Coxon, B. and Schaffer, R. (**1971**). Characterization and quantitative analysis of D-glucose for use in clinical analysis. *Analytical chemistry*, *43*(12), p.1565-1570.

Dhillon, S. (**2010**). Sitagliptin. *Drugs*, *70*(4), p.489-512.

Drucker, DJ. and Nauck, MA. (**2006**). The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *The Lancet*, *368*(9548), p.1696-1705.

Fawcett, J. and Scott, J. **(1960).** A rapid and precise method for the determination of urea J. Chim. Pathol, 13, p. 156.

Foley, J.E. and Jordan, J. **(2010).** Weight neutrality with the DPP-4 inhibitor, vildagliptin: mechanistic basis and clinical experience. *Vascular health and risk management*, *6*, p.541-548.

Fowler, MJ. (**2008**). Microvascular and macrovascular complications of diabetes. *Clinical diabetes*, *26*(2), p.77-82.

Giorgino, F., Laviola, L. and Leonardini, A. (**2005**). Pathophysiology of type 2 diabetes: rationale for different oral antidiabetic treatment strategies. *Diabetes research and clinical practice*, *68*, p.22-29.

Hansotia, T., Baggio, LL., Delmeire, D., Hinke, SA., Yamada, Y., Tsukiyama, K., Seino, Y., Holst, JJ., Schuit, F. and Drucker, DJ. (**2004**). Double incretin receptor knockout (DIRKO) mice reveal an essential role for the enteroinsular axis in transducing the glucoregulatory actions of DPP-IV inhibitors. *Diabetes*, *53*(5), p.1326-1335.

Herrera-Arellano, A., Aguilar-Santamaria, L., Garcia-Hernandez, B., Nicasio-Torres, P. and Tortoriello, J. (**2004**). Clinical trial of Cecropia obtusifolia and Marrubium vulgare leaf extracts on blood glucose and serum lipids in type 2 diabetics. *Phytomedicine*, *11*(7), p.561-566.

International Diabetes Federation (**2011).** Atlas of Diabetes global estimates of the Prevalence of diabetes for 2011 and 2030.

International Diabetes Federation (**2013**). Atlas of Diabetes; Sixth edition 7.

International Diabetes Federation Guideline Development Group (**2014**). Global guideline for type 2 diabetes. *Diabetes research and clinical practice*, *104*(1), p.1.

Ismail-Beigi, F. (**2012**). Pathogenesis and glycemic management of type 2 diabetes mellitus: a physiological approach. *Archives of Iranian Medicine (AIM)*, *15*(4).

Kapadia, K., Somani, G. and Sathaye, S. (**2014**). Inhibition of DPP-IV: A New Therapeutic Approach for the Treatment of Type 2 Diabetes. *Bombay Technologist*, *64*(1).

Karen, B., Mary, E. and Cox, B. **(2010**). Diabetes. Metab Synd Obes, 3, p.363- 372.

Khoo, J., Rayner, C.K., Jones, K.L. and Horowitz, M. (**2009**). Incretin-based therapies: new treatments for type 2 diabetes in the new millennium. *Ther Clin Risk Manag*, *5*(3), p.683-698.

Krentz, AJ. and Bailey, CJ.(**2005**). Oral anti-diabetic agents. *Drugs*, *65*(3), p.385-411.

Lorenzo, C., Wagenknecht, L.E., D'Agostino, RB., Rewers, MJ., Karter, AJ. and Haffner, SM. (**2010**). Insulin Resistance, β-Cell Dysfunction, and Conversion to Type 2 Diabetes in a Multiethnic Population the Insulin Resistance Atherosclerosis Study. *Diabetes Care*, *33*(1), p.67-72.

Mohieldein, H., Khairia, E., Marghoob, H. **(2014).** Lipoprotein (a) and atherogenic indices in Sudanese patients with type 2 diabetes. International Journal of Health Sciences, 8, p.238-246.

Monami, M., Iacomelli, I., Marchionni, N. and Mannucci, E. (**2010**). Dipeptydil peptidase-4 inhibitors in type 2 diabetes: a meta-analysis of randomized clinical trials. *Nutrition, Metabolism and Cardiovascular Diseases*, *20*(4), p.224-235.

Nauck, M.A., Vilsbøll, T., Gallwitz, B., Garber, A. and Madsbad, S., **(2009**). Incretin-Based Therapies Viewpoints on the way to consensus. *Diabetes care*, *32*(suppl 2), p.223-231.

Nauck, MA., Vardarli, I., Deacon, CF., Holst, JJ. and Meier, JJ. (**2011**). Secretion of glucagon-like peptide-1 (GLP-1) in type 2 diabetes: what is up, what is down?. *Diabetologia*, *54*(1), p.10-18.

Nathan, DM., Buse, JB., Davidson, MB., Heine, RJ., Holman, RR., Sherwin, R. and Zinman, B. (**2006**). Management of hyperglycemia in type 2 diabetes: A consensus algorithm for the initiation and adjustment of therapy a consensus statement from the American Diabetes Association and the European Association for the study of diabetes. *Diabetes care*, *29*(8), p.1963-1972.

National Heart Foundation in association with the Faculty of Public Health and Department of Health **(2007)**. Lightening the load: tackling overweight and obesity: a toolkit for developing local strategies to tackle overweight and obesity in children and *adult*.

National Heart Lung and Blood Institute (**2014**). Risk assessment tool for estimating your 10-year risk of having a heart attack.

National Institute for Health and Clinical Excellence **(2009).**http://www.guidance.nice.org.uk/

Neeland, IJ., Turer, AT., Ayers, CR., Powell-Wiley, TM., Vega, GL., Farzaneh-Far, R., Grundy, SM., Khera, A., McGuire, DK. and de Lemos, JA. (**2012**). Dysfunctional adiposity and the risk of prediabetes and type 2 diabetes in obese adults. *Jama*, *308*(11), p.1150-1159.

Peake, M. and Whiting, M. **(2006).** Measurement of Serum Creatinine – Current Status and Future Goals Clin Biochem Rev, 27, p.173-184.

Pratley, R.E. and Salsali, A. (**2007**). Inhibition of DPP-4: a new therapeutic approach for the treatment of type 2 diabetes. *Current medical research and opinion*, *23*(4), p.919-931.

Pratley, RE. (**2008**). current and emerging therapies: an overview of incretin-based therapy in the management of type 2 diabetes. *Journal of the American Academy of Nurse Practitioners*, *20*, p.7.

Rask-Madsen, C. and King, GL. (**2013**). Vascular complications of diabetes: mechanisms of injury and protective factors. *Cell metabolism*, *17*(1), p.20-33.

Rudasingwa, GJ., Amendezo, E. and Twagirumukiza, M. **(2012**). Clinical patterns and complications of African diabetes patients: preliminary data from Kigali University Teaching Hospital, Rwanda. African Journal of Diabetes Medicine, 20, p. 39-42.

Sachin, A., Kumar, O. and Divya, V. (**2009**). Characterisation of streptozotocin induced diabetes mellitus in Swiss albino mice. *Global J Pharmacol*, *3*(2), p.81-84.

Turnbull, F.M., Abraira, C., Anderson, RJ., Byington, RP., Chalmers, JP., Duckworth, WC., Evans, GW., Gerstein, HC., Holman, RR., Moritz, TE. and Neal, BC. (**2009**). Intensive glucose control and macrovascular outcomes in type 2 diabetes. *Diabetologia*, *52*(11), p.2288-2298.

Vasudev, M. and Jann, M. **(2011).** Inpatient Management of Hyperglycemia and Diabetes Clinical Diabetes, 29, p.3-9.

Vilsboll, T., Rosenstock, JM., Yki-Jaervinen, H., Cefalu, WT., Chen, Y., Ling, Y., Meehan, AG., Katz, L., Engel, SS., Kaufman, KD. and Amatruda, JM. (**2009**). Sitagliptin, a Selective DPP-4 Inhibitor, Improves Glycemic Control When-Added to Insulin, with or without Metformin, in Patients with Type 2 Diabetes. In *Diabetes,* 58, p. 158-159.

Wahlfeld, A.W. (**1974**). Triglycerides determination after enzymatic analysis. Methods of Enzymatic Analysis, p.1831-1834.

Wasada, T., McCorkle, K., Harris, VI., Kawai, K., Howard, B. and Unger, RH. (**1981**). Effect of gastric inhibitory polypeptide on plasma levels of chylomicron triglycerides in dogs. *Journal of Clinical Investigation*, *68*(4), p.1106.

Whiting, DR., Guariguata, L., Weil, C. and Shaw, J. (**2011**). IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes research and clinical practice, 94(3), p.311-321.

Whiting, DR., Guariguata, L., Weil, C. and Shaw, J. **(2011**). IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes research and clinical practice, 94(3), p.311-321.

Whitmer, RA., Karter, AJ., Yaffe, K., Quesenberry, CP. and Selby, JV. (**2009**). Hypoglycemic episodes and risk of dementia in older patients with type 2 diabetes mellitus. *Jama*, *301*(15), p.1565-1572.

WHO (**2011**). Integrating traditional medicine with biomedicine towards a patient-centered healthcare system. Chinese Journal of Integrative Medicine, 17(2), pp.83-84.

WHO **(2015a).** Diabetes. Available at http://www.who.int/mediacentre/factsheets/fs312/en.

Wild, S., Roglic, G., Green, A., Sicree, R. and King, H. **(2004**). Global prevalence of diabetes estimates for the year 2000 and projections for 2030. Diabetes care, 27(5), p.1047-1053.

Yki-Järvinen, H. (**2004**). Thiazolidinediones. New England Journal of Medicine, 351(11), p.1106-1118.

Zander, M., Madsbad, S., Madsen, JL. and Holst, JJ. (**2002**). Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and β-cell function in type 2 diabetes: a parallel-group study. The Lancet, 359(9309), p.824-830.

ANNEXS

ANNEX–A: Informed consent and patient information sheet (English version)

My name is Alias Tesfamariam and I am MSc. student in Medical Biochemistry at Addis Ababa University. I am doing a research entitled comparative study between incretin and non-insulin therapies in patients with Diabetes type 2.

The objective of the study is to observe the effect of these two groups of medication on lipid profile, renal function test and fasting blood glucose level and body mass index. If you are agree to participate in the study, about 5 ml of blood will be collected from you or you will allow us to use the sample that you will give for your medical examination and you will be interviewed. During collection of blood, you may feel some discomfort, but this does not produce serious pain. All the data obtained will be kept strictly confidential by using only code numbers and locking the data, only study personnel will have access to the files. Anonymous testing will be undertaken, that is sample will be coded and positive result will not be identified by names. There will be no costs to you as a result of taking part in this study and you are not asked to pay for the laboratory examination. I will give you the result and if your result is clinically significant, I will contact you to the physician for further diagnosis and treatment. Your participation is purely voluntary, and you cannot participate or you can with draw any time after you get involved in the study or you can also jump (decline) to answer some of the questions if you feel uncomfortable. Participation and not participation has no influence on the service you seek to get.

Participant's response: I am free to decline to be in this study, or to withdraw from it at any point and also to jump a question that feels me discomfort. He promised to give the result without cost .My decision as to whether or not to participate in this study will have no influence on my present or future medical service. My signature below indicates that I agree to participate in this study.

Signature of Person Obtaining Consent---------- **Date** ----------

<u>ANNEX –B</u>: ለጥናቱ ተሳታፊዎች የሚሰጥ የፈቃደኝነት ማረጋገጫ ቅፅ (በአማርኛ የተዘጋጀ)

አልያስ ተስፋማርያም የተባልኩ በአዲስ አበባ ዩኒቨርስቲ የድህረ ምረቃ ት/ት ቤት የባዮኬሚስትሪ ተማሪ ነኝ፡በአሁኑ ሰአት በስኳር በሽታ የሚወሰድ መዳኃኒት አማካኝነት ያለው ልዩነት በሚወስዱት ታካሚዎች በደም ዉስጥ ባለ ስብ ፣በሰዉነት ክብደት እና በደም ውስጥ ያለው የስኳር መጠን ላይ የሚያመጣዉን ተጽእኖ ውጤት ለማዎቅ የሚስጥር ቁጥር---------- የጥናቱ ተሳታፊ ፊርማ----------ቀን----------

ዋና ተመራማሪ፡ ኤልያስ ተስፋማርያም

ይህ ጥናት በአዲስ አበባ ዩኒቨርስቲ አስተባባሪነት፤ በስኳር በሽታ የሚወሰድ መዳኃኒት አማካኝነት ያለው ልዩነት በሚወስዱት ታካሚዎች በደም ዉስጥ ባለ ስብ ፣በሰዉነት ክብደት እና በደም ውስጥ ያለው የስኳር መጠን ላይ የሚያመጣዉን ተጽእኖውጤት ለማዎቅ እና የተሻለ የመዳኃኒት ጥቅም ለማግኘት፡፡ በመጠይቁ ላይ የሚሰጠው የእኔ ሙሉ መረጃ በሚስጥር እንደሚያዝ ተነግሮኛል በተጨማሪም ጥናቱ ውስጥ ላለመሳተፍ እምቢ ማለት መብቴ እንደሆነና በማንኛውም ጊዜ ከጥናቱ በራሴ ሁሳኔ መውጣት ጭምር መብቴ መሆኑን ከጥናቱ በመውጣቴ ምንም ዓይት መጉላላት እንደማይደርስብኝ በሚገባ ተገልጾልኛል፡፡ ስለሆነም ሁኔታውን በሚገባ በማጤን በፈቃደኝነት በምርምሩ ላይ ለመሳተፍ ለተመራማሪው ፍቃደኝነቴን ሰጥቻለሁ ፡፤ በተጨማሪም የምሰጠው የደም ናሙና ለ Lipid profile,Fasting Blood Sugar,Renal function test ምርመራዎች ብቻ እንደሚውል ተነግሮኝ ተስማምቻለሁ፡፡ ማንኛውም ያልገባኝን ነገር የመጠየቅ እድል ተሰጥቶኝ በሚገባኝ ቋንቋ መልስ አግኝቻለሁ፡፡ ይህ መረጃ በምንም ዓይነት ከዋናው ተመራማሪ እና ከሀኪም በስተቀር ለማንም አይገለጽም ፡፡ የተሰበሰበው ናሙና ከጥናቱ ዓላማ ውጪ ለሌላ ዓላማ አይውልም ፡፡የጥናቱ ሪፖርት ይፋ በሚሆንበት ግዜ የእርስዎ ስም አይገለጽም ፡፡

ለትብብርዎ በጣም እናመሰግለን!!

ANNEX –C: English version written questionnaire

Addis Ababa University School of Graduate Studies

Department of Medical biochemistry

Questionnaire to be filled by each participant

Interviewer: ---------- **Date of interview**: ----------

I. Identification

1. Code number----------

II. Socio demographic variables

*1 .Age*_____

*2. Weight*_____

3. Height _____

4. Sex:

- Male
- Female

5. Marital status:

- Married
- Single
- Divorced
- Widowed

6. **Educational *status:***

- Illiterate
- 1-6 Grades
- 11-12 grades
- >12+

7. Occupation:

- Have no job
- Private employee
- Government
- Farmer
- House wife

- Retired
- Sex worker/prostitute/
- Student

8. Active smoking:

- Yes
- No

9. Alcohol consumptions:

- Yes
- No

***III.* History of diabetes**

1. Types of diabetics:

- type1
- type 2

2. Duration of diabetes:

- <5 year
- 5-10 year
- >10 year

3. Treatment Regimens:

A) Sulfonylurea
B) Biguanides/metformin
C) Alpha-Glucosidase Inhibitors
D) Thiazolidinediones
E) Incretin
F) If it is Combination therapies list them down in the space provided below

FBS_____

Exclusion criteria'

- Both sexes who are age less than 18 years
- Suffer from HIV/AIDS
- Patients with cancer
- Type 1 diabetes millets
- Pregnant women
- Patients on statin treated

THANK YOU FOR YOUR PARTICIPATION!!!

ANNEX –D: **Amharic version written questionnaire**

የመረ ጃ መሰብሰቢያ መጠይቅ ፎርም/ቅጽ/

አዲስ አበባ ዩንቨርሲቲ የድህረ ምረቃ ት/ት ቤት የባዮ ከ ሚ ሰ ት ሪ

በጥናቱ ተሳታፊ በሆኑ ግለሰቦች የሚሞላ መጠይ ቅፎርም

የጠያቂው ስም_____`ቀን_____መለያ ቁጥር_____

I.የግለሰቡ አ ካላዊ ማህበራዊ እና ኢኮኖሚያዊ ዝርዝር ሁኔታ

1. ዕድሜ____
2. ክብደት____
3. ቁመት_____
4. ጸታ:
    - ሴት
    - ወንድ
5. የጋብቻ ሁኔታ:
    - ያገባ
    - ያለገባ
    - የተፋቱ
    - የትዳር ጓደኛዎ የሞተበዎት
6. የትምህርት ሁኔታ:
    - ያልተማረ
    - ከ1-6 ክፍል
    - ከ7-10ክፍል
    - ከ 11-12ኛ ክፍል
    - ከ12 ክፍል በለይ
7. ስራ:
    - ስራ አጥ
    - የመንግስት ሰራተኛ
    - ገበሬ
    - የቤት እመቤት
    - ሴትኛ አዳሪ
    - የግል ስራ

- ተማሪ

8. ሲጋራ ያጨሳሉ:
   - አዎ አጨሳለሁ
   - አላጨስም
9. መጠጥ ይጠጣሉ:
   - አዎ
   - አልጠጣም

II. የስኳር ህመምን በተመለከተ

1. የስኳር ህመሙ አይነት:
   - አይነት I
   - አይነት II
2. የስኳር በሽታ ካመመዎት ስንት አመት ሆነዎት:
   - ከ5 ዓመት በታች
   - ከ5-10 ዓመት
   - ከ10 ዓመት በላይ
3. የሚዎስዱት የመድሃኒት አይነት:

ሀ) ሳልፈነዩሪያ

ለ) ሜትፎርሚን

ሐ) አልፋ-ግሉኮሲዴዝ ኢንሂቢተር

መ) ታይዛዳዮን

ሠ) የተቀላቀሉ መዲሀኒት

ለትብብርዎ በጣም እናመሰግናለን!