ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Assessment of glycemic control among Type 2 Diabetes Mellitus patients on metformin treatment in Wolaita Sodo University Teaching Referral Hospital, SNNPR, Ethiopia.

By- Eyouel Shimeles (BSc, MSc candidate)

Advisors:

Mistire Wolde (MSc, PhD)

Mekdes Alem (MSc)

A research thesis submitted to the Department of Medical Laboratory Sciences, College of Health Science, Addis Ababa University, in partial fulfillment of the requirements for the degree of Master of Science (MSc) in Clinical Laboratory Sciences (Clinical Chemistry track).

January, 2021

Addis Ababa, Ethiopia

Addis Ababa University

School of Graduate Studies

This is to certify that the thesis prepared by **Eyouel Shimeles**, **entitled: Assessment of glycemic control among Type 2 Diabetes Mellitus patients on metformin treatment in Wolaita Sodo University Teaching Referral Hospital, SNNPR, Ethiopia 2021** and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Clinical chemistry track) complies with the regulations of the University and meets the accepted standards concerning originality and quality.

Signed by the Examining Committee:

External Examiner_____ Signature _____Date _____

Internal Examiner_____ Signature _____ Date _____

Advisor _____Signature _____ Date _____

Advisor _____Signature _____Date _____

_____

Chairman of the Department or Graduate Program Coordinator

ACKNOWLEDGEMENT

I would like to thank God for every single day that I get by his will. I thank Addis Ababa University, College of Health Sciences, and Department of Medical Laboratory Sciences for giving me this chance and cover financial fund.

I would like to forward my deepest gratitude to my advisors Dr. Mistire Wolde and Mekdes Alem for their full guidance, constructive comments, timely advice and valuable suggestions.
My sincerely thanks also go to the clinical Nurses who selected the participants according to the selection criteria and to all participants who will be help me in fulfilling the questionnaire and donate biologic samples.

Finally, I would like to thank the participants for their willingness to participate and Wolaita Sodo University Teaching and Referral Hospital for all the support.

Contents

List of Table

List of Figures

Abbreviations

ADA	American Diabetes Association
BMI	Body Mass Index
CK	Creatinine Kinase
CI	Confidence Interval
DM	Diabetes Mellitus
EDTA	Ethylene Diamine Tetra acetic Acid
GLP-1	Glucagon Like Peptide 1
HbA1c	Glycated Haemoglobin
HDL-c	High density Lipoprotein Cholesterol
IDF	International Diabetes Federation
IR	Insulin Resistance
LMIC	Low and Middle Income Countries
Mg/dL	Milli gram per decilitre
NCD	Non Communicable Disease
NCEP	National Cholesterol Education Program
NIDDM	Non-Insulin Dependent Diabetes Mellitus
SNNPR	Southern Nations Nationalities and Peoples Region
SOP	Standard Operating Procedure
SPSS	Statistical Package for Social Science
TC	Total Cholesterol
TG	Triglyceride
T2DM	Type 2 Diabetes Mellitus
WHO	World Health Organization
WSUTRH	Wolaita Sodo University Teaching Referral Hospital

Abstract

Background: Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Glycemic control is considered as the principle helpful objective for counteraction of organ harm and different intricacies of diabetes. The first line of remedy for T2DM is metformin, which lack relative facet outcomes and its brilliant affected person tolerance.

Objective: To assess glycemic control among Type 2 Diabetes Mellitus patients on metformin treatment in Wolaita Sodo University Teaching Referral Hospital, SNNPR, Ethiopia.

Methods: A Hospital based Cross sectional study design was carried out among diabetic adults from February 2021 to April 2021 at Wolaita Sodo Hospital, Southern Ethiopia. A total of 140 study participants were selected during follow up by consecutive sampling technique. Structured questionnaires were used for socio-demographic, and anthropometric data collection. In addition, after overnight (8-12 hours) fasting 5 ml of blood sample was collected from each participant by serum separator, (SS) tube, and Fasting blood sugar (FBS), HbA1c and lipid profiles were measured. Data were analyzed by using Epi data version 3.1 and SPSS version 21.0 statistical software. Descriptive statistics (Frequencies, mean, SD, percentage) were used to explain study population in relation to relevant variables. Logistic regression was used for data comparison, **P** value <0.05 were accepted as statistically significant, and finally the result was presented using text, tables, charts and graphs.

Result: The age of study participant**s** ranged from 25-80 years with an overall mean ± SD age of 48.4±10.6 years. Of the total 134 patients, 58% (78)had a mean HbA1c 7.9% resulting in poor glycemic control. We identified that patients with age range between 41-55 years (p=0.005) were poorly managed their blood glucose level compared to the other age groups under study. Poor glycemic control were associated with age (AOR:8.87, 95% CI 1.9-40),Triglyceride (AOR:0.27, 95% CI 0.08-0.89), metformin taking (AOR: 0.005,95%CI 0.00-0.04) and comorbidity (AOR: 0.21, 95%CI 0.05-1.2).

Conclusion: This study shows nearly two-third (58.2%) of diabetic patients attending Wolaita Sodo Hospital ,Southern Ethiopia had poor glycemic control. The variables found to influence the outcome of glycemic control in the present study were age, comorbidity, Triglyceride and dose of metformin taking.

Key words: Diabetes Mellitus, Glycemic Control, Glycated Hemoglobin

1. Introduction

1.1 Background:

Type 2 diabetes mellitus (T2DM) is a metabolic disorder and typically results from excess of caloric intake over energy expenditure (1)**.**

In T2DM, insulin obstruction adds to expanded glucose creation in the liver and diminished glucose take-up in muscle and fat tissue at a set insulin level. Likewise, β cell brokenness brings about diminished insulin discharge, which is deficient for keeping up normal glucose levels. Both insulin opposition and β cell dysfunction happen right off the bat in the pathogenesis of T2DM, and their basic significance has been checked longitudinally in advancing from typical glucose resilience to impeded glucose resistance to T2DM (2)**.**

During the characteristic course of diabetes, β -cells emit extra insulin in the beginning stage of insulin obstruction and the insulin levels at first increase. This increase in insulin emission in reality actually speaks to a general deficiency of insulin, on the grounds that from the get-go over the span of the regular history, β -cell work begins to crumble. This pathophysiological decrease or deformity adds to the reformist idea of the infection in long-standing sort 2 diabetes (3)**.**

Patients with type 2 diabetes have serious level of hyperglycemia and expanded danger of microvascular difficulties, tangible, neuropathy, myocardial localized necrosis, stroke, macrovascular mortality, and all-cause mortality (4) **.**

There are a few danger factors for the movement of Type 2 DM (T2DM) including family ancestry, corpulence, ongoing actual dormancy, race or nationality, history of hindered fasting glucose, impeded glucose resilience, HbA1c 5.7% to 6.4% (38.8mmol/mol to 46.4mmol/mol), hypertension, anomalous high-thickness lipoprotein cholesterol and additionally raised fatty oil levels. The span of diabetes, way of life, educational status, age, number of drugs, bleakness, financial factors and type of medication inclusion, are hazard factors for continued poor

glycemic control. People in danger of poor glycemic control may require explicit intercessions to accomplish ideal glycemic control (5)(6)**.**

Glycemic control is considered as the principal helpful objective for counteraction of organ harm and different intricacies of diabetes. Hence, accomplishing glycemic control is a basic metabolic objective since hyperglycemia adds to the progression of diabetes mellitus by influencing both ß-cell capacity and insulin affectability (7).

The Fasting Plasma Glucose (FPG) and the Oral Glucose Tolerance Test are the most commonly known T2DM diagnostic tests (OGTT). Diagnostic tests are widely used for both FPG (diagnostic of diabetes at plasma glucose level ≥ 126 mg/dL or 7.0 mmol/L) and 2-hour OGTT (diagnostic of diabetes at plasma glucose level ≥ 200 mg/dL or 11.1 mmol/L). Low costs and the popularity of automated laboratory equipment are the benefits of FPG. The OGTT has long been known as one of the diagrams, though (1)**.**

Blood HbA1c is a favorable diagnostic tool for the following reasons. First, HbA1c measurements can be carried out at any time and do not require preparation by tested subjects. Second, its intraindividual biological variability is low, hence with high reproducibility (1)**.**
Glycosylated hemoglobin (HbA1c) is the essential objective of glycemic control. HbA1c is framed by non-enzymatic covalent expansion of glucose moieties to hemoglobin in red cells. Dissimilar to blood glucose levels, HbA1c is the file that shows the normal blood glucose during the previous 3 months and is minimal influenced by everyday varieties (8)**.**

To sluggish T2DM development and assist sufferers attain glycemic targets, professional pointers stress the significance of complete sickness management. Combined with life-style modification, a number of oral anti-hyperglycemic retailers are endorsed to assist sufferers attain glycemic objectives (9)**.**

Metformin is an antihyperglycemic which in patients with type 2 diabetes improves glucose tolerance, reducing both basal and postprandial plasma glucose. Its pharmacological mechanisms of action vary from those of other oral antihyperglycemic agent groups. Metformin decreases the

production of hepatic glucose, decreases glucose intestinal absorption, and increases insulin sensitivity by increasing the absorption and utilization of peripheral glucose (10)**.**

The first line of remedy for T2DM is metformin, a remedy that is liked for its relative lack of facet outcomes and its brilliant affected person tolerance. However, due to variations in character genetic profiles, metformin does no longer function equally nor optimally in all patients, main to a discount in the drug's efficacy and security (11). Metformin increases insulin sensitivity, resulting in reduced hepatic glucose output and increased glucose uptake in muscle (12)**.**

The main mechanisms include anorexiogenesis, reduction of intestinal carbohydrate absorption, inhibition of hepatic gluconeogenesis, as well as increased glucose uptake by peripheral tissues. Reduced appetite is a useful action of metformin, contributing to weight loss, which is beneficial, given that the vast majority of patients are obese(13)**.**

1.2 Statement of the Problem

Diabetes is now a disease of major concern both globally and regionally and is a leading cause of death in most countries (14).

Between 1980 and 2014, the number of people living with diabetes mellitus doubled worldwide. Between 2010 and 2030, the number of adults with diabetes mellitus in industrialized countries is expected to rise by 20%, whereas in developing countries it is expected to rise by 69% (2).

The World Health Organization (WHO) reported that 108 million individuals had diabetes in 1980, and that number has increased fourfold by 2014, with a global incidence of 151 million people (15) According to the International Diabetes Federation (IDF), 10.9 million Americans, or 26.9% of those aged 65 and more, had diabetes in 2010, while roughly 215,000 people younger than 20 years had diabetes (type 1 or type 2) in the United States. Type 2 diabetes used to be a dangerous condition (16).

Between 2000 and 2035, the increasing of type two diabetes in South Asia is estimated to be over 150%. There are greater than 138.2 million people with diabetes in the Western Pacific, the world's most populated area, and the wide variety should develop to 201.8 million via 2035 (14).

In China alone, the age-standardized occurrence of diabetes improved to 5.04% for each boys and girls in 2017. Due to its large population, there had been an estimated 3,338,131 new cases and 153,184 deaths of diabetes in China in 2017, accounting for 14.55% and 11.18% of all new cases and all deaths of diabetes worldwide, respectively (17).

Diabetes incidence has increased by 129.0 % in the WHO Africa region, demonstrating that LMICs have had a greater rapid upward thrust in diabetes incidence than high-income global areas in particular. According to the International Diabetes Federation, diabetes incidence in Sub-Saharan Africa is expected to more than double by 2035(18).

The Lancet Diabetes and Endocrinology Commission stated in 2015 that the complete rate of diabetes in sub-Saharan Africa used to be US$19.4 billion or 1.2% of the combined gross domestic product (GDP), with direct prices of $10.8 billion (55.6%) which consist of expenditure on diabetes care (purchase of medicines), sanatorium stays, and remedy of (19).

Ethiopia, close to the rest of the SSA nations, is experiencing with increased prevalence, a major diabetes burden, Mortality and risks, as well as life Disabilities that endanger (20)**.**
Previous findings in Ethiopia also reported that the rate of poor glycemic control was high most importantly due to non-compliance to existing medications(20)**,**(21)**,**(22)**,**(23)**.**

Despite the prevalence of type 2 DM is increasing rapidly in Ethiopia, data regarding glycemic control is scarce, and little is known about the factors contributing to poor glycemic control (24)**.**

Early identification and intervention of at-risk subjects can prevent the ongoing of diabetes and control the progression to other chronic diseases, which will bring lots of benefits to human health and reduce the individual and societal burden of related diseases. In view of the above reasons, there are a rising number of anthropometric, biochemical indicators applied to predict diabetic complications.

1.3 Significance of the study

The epidemic of diabetes mellitus and its complications poses a major global health threat (2).

In the study area, the determinants of the glycemic control have not yet been studied in the communities. The aim of this study is to assess Predictive factors of poor glycemic control among Type 2 Diabetes Mellitus patients in Wolaita Sodo University Teaching Referral Hospital, SNNPR, Ethiopia.

By showing existing gaps, the results will serve as baseline data for program managers and decision makers. It enables hospitals to modify prevention guidelines and to evaluate its implementation process by all health professionals. The study will also aid to secure patients right to be prevented harms.

2. Literature review

2.1 Overview of T2 DM

Type 2 DM was first described as a component of metabolic syndrome in 1988 and formerly known as non-insulin dependent DM (23)**.**

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels (24)**.**

T2DM can be considered one of the chronic diseases of greater impact for the public health system. In addition to causing a high degree of morbidity and mortality, the metabolic control of diabetes and the treatment of its complications have a high cost for health services (25)**.**

T2DM has become an observably global public health problem. Analysis of recent statistical data reveals that T2DM has several new epidemiological characteristics. Firstly, diabetes keeps a steady increase in developed countries, such as United States and Japan. And it is worthy of note that T2DM has become a serious issue at an alarming rate in developing countries (26)**.**

This structure of diabetes, which money owed for 90–95% of these with diabetes, earlier referred to as non-insulin structured diabetes, type II diabetes, or adult-onset diabetes, encompasses men and women who have insulin resistance and normally have relative (rather than absolute) insulin deficiency At least initially, and frequently all through their lifetime, these folks do no longer want insulin remedy to survive. Most sufferers with this structure of diabetes are obese, and weight problems itself motives some diploma of insulin resistance. Patients who are now not chubby with the aid of typical weight standards can also have an improved proportion of physique fats dispensed predominantly in the stomach region. Ketoacidosis seldom takes place spontaneously in this kind of diabetes; when seen, it typically arises in affiliation with the stress of some other ill- ness such as infection. This shape of diabetes regularly goes undiagnosed for many years due to the fact the hyperglycemia develops step by step and at in the past tiers is regularly no longer extreme ample for the affected person to word any of the basic signs and symptoms of diabetes. Nevertheless, such sufferers are at expanded chance of growing macrovascular and microvascular complications (27)**.**

2.1.1 Pathophysiology

The uptake of glucose from the blood into most cells of the body, especially liver, muscle, and adipose tissue is regulated by the principal hormone, insulin (28)**.**

NIDDM is characterized by using insulin insensitivity as end result of insulin resistance, declining insulin production, and eventual pancreatic beta-cell failure. This leads to reduction in glucose transport into the liver, muscle cells, and fats cells. There is accelerated breakdown of fats with hyperglycemia. The involvement of impaired alpha-cell characteristic has these days been identified in the pathophysiology of type two DM (29)**.**

When β cells bear IR, at first there is insulin hyper-secretion which compensates for the lack of hormonal action. Hyperglycemia solely manifests when there exists a relative insulin hypo-secretion to the glucose stimulus. Hyperinsulinemia is at the beginning successful of preserving ordinary fasting and postprandial glycaemia. This stage would be related with accelerated tiers of free fatty acids (FFA) in the overweight IR patient(30)**.**

The transcription element 7-like two (TCF7L2) gene has given the affect to be extra applicable inside the genetic susceptibility to DM2, given that a polymorphism of this gene has been located in quite a few ethnic group of DM2 patients. This issue is related to a decreased response to glucagon-like peptide-1 (GLP-1) due to the fact GLP-1 expression in enteroendocrine cells is regulated through TCF7L2, which may have as a remaining consequence, a failure in β cell proliferation and in insulin secretion; thus, versions of the TCF7L2 gene would make a contribution to the threat for DM (30)**.**

Glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), mutually referred to as incretins, act on the pancreatic islet. GLP-1 is greater important, and acts each on β cells to fortify insulin secretion and on α cells to suppress glucagon secretion. Therefore, the β-cell response to GLP-1 after meal ingestion has acquired to be deficient, as mentioned after intravenous administration of GLP-1 beneath managed conditions. This deficient response is in accordance to a mannequin of international deficiency in β-cell responsiveness to a number of secretagogues (E.g., sulfonylurea antidiabetics, aminoacids, and β-adrenoreceptor agonists) (31)**.**

2.1.2 Risk factors

Clinical expression of the disorder requires both genetic and environmental factors. One theory concerning its etiology is that it is the result of the evolution of a thrifty genotype that had survival benefits in the past but is detrimental in the current environment. Hyperglycemia in type 2 diabetes results from absolute or relative insulin deficiency (32)**.**

A wide variety of lifestyle factors are also of great importance to the development of T2DM, such as sedentary lifestyle, physical inactivity, smoking and alcohol consumption. Substantial epidemiological studies have shown that obesity is the most important risk factor for T2DM, which may influence the development of insulin resistance and disease progression (26)**.**

2.1.3 Complication

Diabetic complications associated with hyperglycemia impair the metabolism of carbohydrates, fats, proteins and electrolytes, all of which can disrupt the vascular system. In type 2 DM, there is a strong association between chronic inflammation and delayed wound healing, prolonged infection, atherosclerosis, insulin-resistance, and increased immune-cytokine production (33)**.**

The one or more of the following are caused by the damage of small blood vessels that bring about to a microangiopathy, Diabetic cardiomyopathy Diabetic nephropathy Diabetic neuropathy Diabetic retinopathy Diabetic encephalopathy (28)**.**

The one or more of the following are caused by macrovascular disease: Cardiovascular disease, to which accelerated atherosclerosis, is a contributor. Coronary artery disease leading to angina or myocardial infarction ("heart attack") cerebrovascular disease and peripheral vascular disease, which often lead to morbidity and mortality (25)**,**(28)**.**

2.2 previous study on associated factors of Glycaemic Control

Kaithala C etal in India 2015,showed dyslipidemia in good glycemic control group (HbA1c ≤ 7%) was found to be less compared to that of poor glycemic control group (HbA1c > 7%) (34)**.**
A study done by M. Khattab et al 2010, using a systematic random sample of 917 patients Of the total 917 patients, 65.1% had HbA1c ≥7%. In the multivariate analysis, increased duration of

diabetes (7 years vs. ≤7years) (OR=1.99, P≤.0005), not following eating plan as recommended by dietitians (OR=2.98, P≤.0005), negative attitude towards diabetes, and increased barriers to adherence scale scores were significantly associated with increased odds of poor glycemic control (35)**.**

Benoit et al in Longitudinal observational data in San Diego addressed 573 Patients had a mean age of 55 years, 69% were female, the mean duration of diabetes was 7.1 years, 31% were treated with insulin, and 57% were obese. American Diabetes Association (ADA) recommendations for blood pressure and total cholesterol were met by 71% and 68%, respectively. Results of the mixed effects model showed that patients who were uninsured, had diabetes for a longer period of time, used insulin or multiple oral agents, or had high cholesterol had higher A1C values over time indicating poorer glycemic control. The younger subjects also had poorer control (36)**.**

A cross-sectional study report of Kakade AA et al 2018 in Two hundred twenty patients of Type II diabetes mellitus revealed that statistically significant difference (P = 0.044) was found between patients with good and poor glycemic control in relation with BMI (P = 0.044), central obesity (P < 0.001), dyslipidemia (P < 0.001) and diabetes self-care practices glucose management (P = 0.003), dietary control (P = 0.006), sum scale (P = 0.028). Majority of Type II diabetic patients had poor glycemic control. Factors affecting glycemic control included BMI, central obesity, dyslipidemia and diabetes self-care practices (glucose management and dietary control (8)**.**

A study done by Bukhsh et al 2018 in Pakistan, stated that majority of the sample was >45–60 years old (48.8%), suffering from type 2 diabetes mellitus for <5 years (49.5%) and had poor glycemic control (HbA1C≥7%; n=181 participants). Disease knowledge was significantly associated ($p<0.05$) with patient's gender, level of education, family history of diabetes, nature of euglycemic therapy, and glycemic control. Correlation matrix showed strongly inverse correlations of DKQ with Glycated hemoglobin levels ($r=-0.62$; $p<0.001$) and strongly positive with DSMQ sum scale ($r=0.63$; $p<0.001$). PWD having university-level education ($\beta=0.22$; 95% Confidence Interval (CI) 0.189, 0.872; $p<0.01$), doing job ($\beta=0.22$; 95% CI 0.009, 0.908];

p=0.046), and use of oral hypoglycemic agents in combination with insulin (β=−0.16; 95% CI [−1.224, −0.071]; p=0.028) were the significant predictors for disease knowledge (37)**.**

A descriptive cross sectional study done by Nduati etal 2016 in Nairobi, shows out of 149 participants total of 122(81.6%) had poor glycemic control with a mean HBA1C of 9.1, 90.6% having elevated FBS, 37.6% with elevated T-Chol and 60.4% having high LDL levels. Twenty four percent had moderately increased UACR while 11.4% had severely increased UACR. Gender (OR3.029, 95%CI: 1.287–7.129, p=0.010), FBS (OR=8.14, 95%CI; 2.541-26.0810, p=0.001) and using drugs for other co-morbidities OR=2.519, 95%CI; 1.009-6.288, p=0.035) were associated with glycemic control. There is a high burden of poor glycemic control among T2DM patients in Mathari National Teaching and Referral Hospital especially women (38)**.**

Hospital-based cross-sectional study by Kibirige et al 2017 showed that the median (interquartile range) HbA1c level was 9% (6.8%–12.4%). Suboptimal glycemic control was noted in 311 study participants, accounting for 73.52% of the participants. HbA1c levels of 7%–8%, 8.1%–9.9%, and ≥10% were noted in 56 (13.24%), 76 (17.97%), and 179 (42.32%) study participants, respectively. The documented predictors of suboptimal glycemic control were metformin mono therapy (odds ratio: 0.36, 95% confidence interval: 0.21–0.63, p<0.005) and insulin therapy (odds ratio: 2.41, 95% confidence interval: 1.41–4.12, p=0.001) (39)**.**

A cross-sectional study conducted in Gadarif, eastern Sudan 2018, enrolled a total of 339 patients and the mean age of the participants was 54.8 (12.8) years. Approximately more than two-thirds (n = 243, 71.7%) of the participants were using oral glucose control agents. A round one-fifth (22.1%) of the participants were using insulin and only 6.2% of them were using both insulin and oral glucose control agents. The rate of poor glycemic control was 71.9%. In logistic regression analyses, duration of diabetes, medications used, and the triglycerides were not associated with poor glycemic control. However, being unmarried (OR = 3.64, 95% CI 1.21–10.90), adding sugar to the drinks (OR = 1.84, 95% CI 1.11–3.05, P = 0.017) and high cholesterol level (OR = 1.01, 95% CI 1.01–1.02.) were associated with poor glycemic control. In summary the rate of uncontrolled type 2 diabetes mellitus was considerably high especially among being unmarried patients and patients who were adding sugar to the drinks(40)**.**

Kamuhabwa R A et al in Dareselam 2014, the cross-sectional study showed that the proportion of poor glycemic control increased with age. A significantly high proportion of poor glycemic control was observed in patients who had had the disease for more than 20 years since diagnosis. Factors associated with poor glycemic control included lack of health insurance, using more than one oral hypoglycemic agent, normal body mass index, obesity, and non-adherence to diabetic medications (7)**.**

A cross sectional study conducted by Woldu MA et al 2014 in Ambo showed that the most frequent comorbidity observed over type 2 diabetes was hypertension 41(40.6%) followed by renal disease and dyspepsia with 5 and 3 patients respectively. Furthermore, patients who had hyperlipidemia and peripheral neuropathy as a comorbidities are 5 and 579 more prone to develop poor blood glucose control compared to patients with no comorbidities with 95% C.I of (1.145-20.462 and 116.8-2870), respectively (22)**.**

A total of 228 type 2 diabetes in hospital based cross-sectional study recruited in a study conducted by Ginenus F etal2018 in Nekemet stated that Age, exercise, level of education, duration of the treatment, and smoking were significantly associated with poor glycemic control (21)**.**

Similarly Institution-based cross-sectional study conducted in 2016 by Yerra Rajeshwar et al in Mekelle indicated that age, hypertension and non-adherence to diabetes self -management behaviors were independent predictors of poor glycemic control (41)**.**

2.3 Conceptual Frame Work

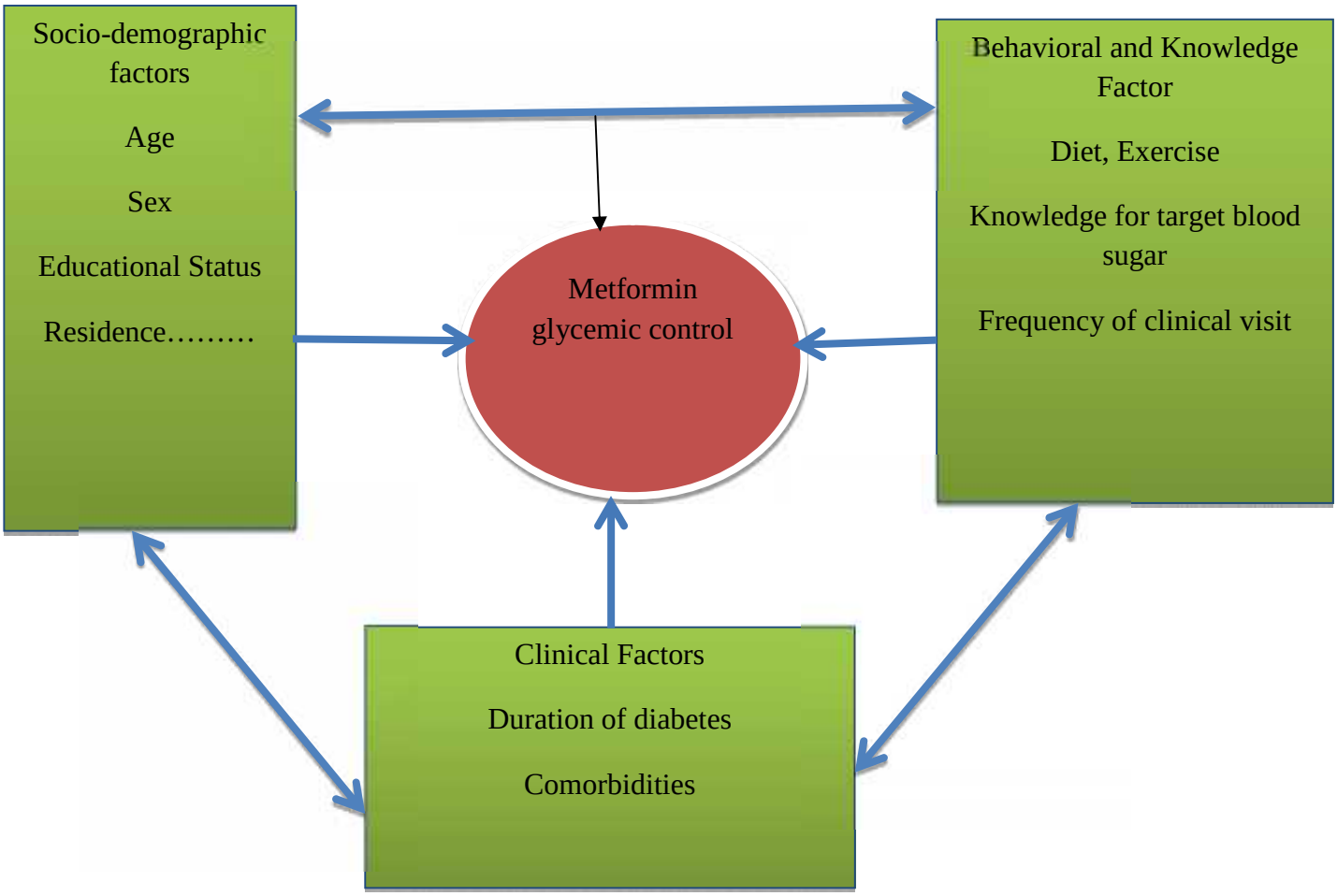


Figure 1 Conceptual frame work developed to show association of dependent and independent factors

3. Objectives

3.1 General Objective

To asses glycemic control among Type 2 Diabetes Mellitus patients on metformin treatment in Wolaita Sodo University Teaching Referral Hospital, SNNPR, Ethiopia from February, 2021 to April, 2021.

3.2 Specific objectives

- To determine glycemic control of T2DM on Metformin among T2DM patients attending DM clinic at Wolaita Sodo University Teaching Referral Hospital, SNNPR, Ethiopia
- To identify associated factors contributed for glycemic control while taking metformin treatment among T2DM patients attending DM clinic at Wolaita Sodo University Teaching Referral Hospital, SNNPR, Ethiopia

4. Materials & Methods

4.1. Study area

The study was conducted at Wolaita Sodo University Teaching and Referral Hospital which is situated at southern Ethiopia, Wolaita Zone, Sodo town and 329 kilometers from Addis Ababa, the capital city of Ethiopia. It was serving people in catchment's area of three million people including neighboring Dawuro zone, Gamo-gofa zone, and Kambata Tambaro one. The former 'Otona Hospital' the current 'Wolaita Sodo University Teaching and Referral Hospital' was established in 1920 E.C as small clinic by SIM (Sudan Interior Mission). The hospital served for about 50 years as primary hospital and then become general hospital for 30 years. In 2004 E.C, Wolaita Sodo University takeover the hospital as a teaching and referral hospital with academic, research and community service responsibilities. The hospital organized in department, case-teams and working hours are according to BPR standard. There are seven case teams, OPD, Emergency, Inpatient, Delivery, Dentistry and Ophthalmology. The hospital has 200 beds for inpatient service which is on medical, surgical gynecology and obstetrics ward (42)**.**

4.2. Study design and period

Hospital based cross sectional study was conducted to assess glycemic control among Type 2 Diabetes Mellitus patients on metformin at WSUTRH , who were enroll from February 2021 to April 2021.

4.3. Population

4.3.1. Source population

The source population was all T2 diabetes patients at WSUTRH.

4.3.2. Study Population

The study population were all patients with type 2 diabetes mellitus who presented at a diabetic clinic during the data collection period and those who fulfilled the eligibility criteria.

4.4. Eligibility criteria

4.4.1. Inclusion criteria

- Patients who had at least three months consecutive follow up will be included
- Who are on metformin treatment at least 6 month
- Reside for at least 3years

4.4.2. Exclusion criteria

- Pregnant women
- Who are taking insulin

4.5. Study variables

4.5.1. Dependent variable:

- Metformin base glycemic control

4.5.2. Independent variables:

- Socio demographic factors (Age, marital status, educational status)
- Clinical related factors (duration of diabetes, comorbidities, WHR, BMI)
- Medication related factors
- Clinical chemistry test (HbA1c, Lipid profile)

4.6. Sample size calculation and Sampling method

4.6.1. Sample size calculation

A single population proportion formula was used to estimate the minimum sample size required for the study, with an elevated FBS 90.6% adopted from a previous study which was conducted in Nairobi.

$$N = \frac{Z^2 pq}{d^2}$$

Where Z confidence level 95%, d margin of error 5%, p expected proportion in population based on previous study.

$$N = \frac{Z^2 pq}{d^2} = \frac{1.96^2 \times 0.91 \times 0.09}{0.05} = 126$$

P=0.91..... Nduati etal 2016(38)

Total sample size 140 with 10% contingency.

4.6.2. Sampling Method:

All T2 DM patients who attend the diabetic clinic during the study period and are eligible for the study were enrolled. Consecutive sampling technique was applied to recruit the required sample sizes. Study participants interviewed up on their exit from diabetic clinic.

4.7. Measurement and Data collection

4.7.1. Data collection procedure:

The diabetic patients were interviewed with an administered questionnaire that was originally prepared in English and then translated to Amharic language questionnaire mainly consisted of closed and open-ended questions and delivered to eligible subjects after consent is taken to collect data face to face interview, and Physical measurements obtained by trained clinical nurses. Height and weight were measured by using a seca stadiometer and a seca weight floor scale (GMPH &CO.KG, Germany) which was placed on flat floor. During the measurement of height and weight the study subject asked to remove her/his shoes, hats and any bulky clothing and the subject looking straight ahead, face forward and the readers eye at the level of the head piece. Then the height and weight were recorded (34,35). The body mass index (BMI) was calculated as weight divided by height square (kg/m^2). According to the WHO classification of BMI can be underweight, < 18 kg/m^2; normal weight, > 18.5 – 24.9 kg/m^2; overweight, > 25.0 – 29.9 kg/m^2 and obese > 30 kg/m^2 (36).

Blood pressure (BP) was measured by using mercury sphygmomanometer (Henry Schein inc. Melville, NY, USA) and stethoscope from left upper arm and positioned at the heart level. After five minutes of rest, three consecutive blood pressure readings were taken at interval of at least one minute and the mean BP taken. (37).

Furthermore, medical records /charts of Hypertensive participants were reviewed to obtain clinical data such as focusing on socio-demographic data, and duration of Hypertension.

The Cut off value for TC ≥ 200 mg/dl, TG ≥ 150 mg/dl, HDL< 40 mg/dl, and LDL ≥ 130 mg/dl, by the United States National Cholesterol Education Program, Adult Treatment Panel (NCEP-ATP) III guidelines (43)**.**

4.7.2. Laboratory analysis

4.7.2.1Specimen collection and processing

We were after obtaining written consent from all subjects who were included in the study and by giving detailed information about the study, instruct the study subject about the procedure and the site was select preferably at the median cubital vein. Warming the arm with hand or hanging

the hand down made it easier to see the veins. The area was palpated to locate the anatomic sites. Apply a tourniquet, about 4–5 finger-widths above the select vein puncture site. The sites were disinfect using 70% alcohol swaps for 30 seconds in a circular motion from inside to outside fashion and allow to dry completely for 30 seconds. Then, the needle was entering swiftly at a 30-degree angle.

About 5 ml of venous blood was collected aseptically from the median cubital vein from each study participant by trained Laboratory Technologists in the morning after 8:00 hours of the overnight fast. The blood sample was dispensed into jell coated serum separator test tube or plain tube and EDTA tube label with a unique ID number. The collected blood sample was left for 30 minutes to facilitate clotting at room temperature.

Then the clotted blood samples were centrifuged for 5 minutes at 3000 revolutions per minute (rpm) to separate serum from formed elements. The fasting blood glucose and lipid profiles were analyzed by Mindray BS-200 and HbA1c analyzed by Siemens dimension EXL-200. However, the serum was kept in Refrigerator at -20 ^{0}C by Nunc tubes still the time of analysis greater than Seven days.

4.7.2.2. Principles of Laboratory analysis and procedures

Biochemical tests such as HbA1c are analyzed by Siemens dimension EXL-200, fasting blood Glucose; Lipid profile and Creatinine level are analyzed by using Mindray BS-200 fully automated clinical chemistry analyzer according to the manufacturer's instructions and procedures in WSUTRH laboratory. The instrument Mindray BS-200 chemistry analyzer is calibrated using Autocal and quality control samples both normal (Humatrol N) and pathological (Humatrol P) are run each day before running Patients samples.

1. Measurement of serum total cholesterol (TC)

Principle

Cholesterol is determined by a timed-endpoint method. Cholesterol esters are hydrolyzed to free cholesterol by cholesterol ester hydrolase (CE). The free cholesterol produced is oxidized by cholesterol oxidase (CO) to cholest-4-ene-3-one with the simultaneous production of hydrogen

peroxide, which oxidatively couples with 4-aminophenazone and phenol in the presence of peroxidase to yield a chromogen. The absorbance is measured at 400 nm.

The reaction sequence is as follow:

Cholesterol ester + H_2O $\xrightarrow{CHE}$ Cholesterol + FFA

Cholesterol + O2 $\longrightarrow$ 4-Cholestinone + H_2O_2

2 H_2O_2 + 4-aminoantipyrine +phenol $\xrightarrow{POD}$ Quinonemine dye + $4H_2O$

Reagent

The reagents are standard and ready for use on automated analyzer. Enzymatic assay was adjusted at 400 nm in wavelength, 1 cm in optical path, 37 ^oC in temperature and measurement done against reagent blank. PIPES-200mmol/L(PH-7.0),Sodium cholate-1mmol/L, Cholesterol ester >250U/L, Cholesterol oxidase>250U/L,Phenol-4mmol/L->1KU/L,4-aminoantipyrine-0.33mmol/L,Phenol-4mmol/L,Non-ionic surface-2 g/l, Biocides.

Procedure: Samples, standard and reagent blank were pre-incubated for 5 minutes at 37 °C. Samples (10 µL) or standard (10 µL) and reagent blank (1000 µL) were pipetted into cuvette and mixed thoroughly by inversion. The cuvettes were inserted into the cell holder and stopwatch was started to count. The absorbance of sample, standard and the reagent blank was measured at 400 nm within 60 minutes. Finally the absorbance of the sample (ΔA sample) and the standard (ΔA standard) against the reagent blank was calculated.

$$TC(mg/dl) = \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} * C \text{ Standard}$$

2. Measurement of serum Triglyceride (TG)

Principle

Triglyceride (TG) level is estimated by a timed-endpoint method. TG measured using a series of coupled reactions in which triglycerides are hydrolyzed to produce glycerol and free fatty acids by the action of enzyme lipase. The glycerol formed phosphorylated to glycerol-3-phosphate by

glycerol kinase. The glycerol-3-phophate is oxidized by glycerol phosphate oxidase producing dihydroxyacetone phosphate and hydrogen peroxide. Then, Peroxidase catalyzes the redox-coupled reactions of H_2O_2 with 4-aminoantipyrine (4-AAP), producing a bright purple color. The absorbance measured at 540 nm.

The reaction sequence is as follows:

Triglycerides + $3H_2O$ $\xrightarrow{\text{LPL}}$ Glycerol + fatty acid

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

Glycerol -3-phosphate +O_2 $\xrightarrow{\text{Glycerol phosphate oxidase}}$ DHAP +H2O2

H_2O_2 + 4-aminoantipyrine $\longrightarrow$ Quinonemine + H_2O

Reagents: The reagents are standard and ready for use on automated analyzer. Enzymatic assay and measurement was done at 540 nm wavelengths, 1cm optical path, and 37 ^{o}C temperature against reagent blank.

Reagent(R): phosphate buffer (PH 7.5)-100mmol/l,LPL-1500 U/L,GK-800 U/L,G-3-P oxidase -4000 U/L, Peroxidase -440 U/L, Phenol – 7.5 mmol/L, 4-aminoantipyrine – 0.7 mmol/L,ATP – 0.3mmol/L.

Procedure: Samples, standard and blank were pre-incubated for 5 minutes at 37 °C. Reagent blank (1000 µL) and samples (10 µL) or standard (10 µL) was put into cuvette and mixed gently by inversion. The cuvettes were inserted into the cell holder and stopwatch started to count. The absorbance of sample, standard and blank was measured at 540 nm. Finally, the absorbance of the sample (ΔA sample) and the standard (ΔA standard) against the reagent blank will be calculated.

$$TG(\text{mg/dl}) = \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} * \text{C Standard}$$

3. Measurements of serum high density lipoprotein cholesterol (HDL-C)

Principle

VLDL-c and LDL-c from serum are precipitated by phosphotungstate in the presence of magnesium ions. After removal by centrifugation, the clear supernatant containing HDL fraction and their cholesterol content was determined enzymatically.

The reactions are as follows:

Cholesterol ester + H_2O $\xrightarrow{\text{chol.estarse}}$ Cholesterol + FFA

Cholesterol + ½ O_2 + H_2O $\xrightarrow{\text{chol. Oxidase}}$ Cholestenone + H_2O_2

2 H_2O_2 + 4-Aminoantipyrine + DSBmT $\longrightarrow$ Quinonemine + $4H_2O$

Reagents

Reagents are standard and ready for use on automated analyzer. Enzymatic assay was done at 593 nm wavelength, 1 cm optical path, 37 °C temperature and measurement was done against reagent blank. PIPES-200mmol/L(PH-7.0),Sodium cholate-1mmol/L, Cholesterol ester >250U/L, Cholesterol oxidase>250U/L,Phenol-4 mmol/L->1KU/L, 4-aminoantipyrine-0.33 mmol/L,Phenol-4 mmol/L, Non-ionic surface-2 g/l, Biocides.

Procedure

Reagent blank, samples and calibrator were pre-incubated for 5 minutes at 37 °C. Reagent blank (10 µL distilled water and 750 µL enzymes) and samples (10 µL samples and 750 µL enzymes) or calibrator (10 µL calibrator and 750 µL enzymes) was pipetted into cuvette and mixed gently by inversion. The cuvettes were inserted into the cell holder and stopwatch were started to count. The absorbance of sample, standard and the reagent blank were measured at 593 nm after 5 minutes. Finally the absorbance of the samples (ΔA sample) and the calibrator (ΔA standard) against the reagent blank was calculated.

$$\text{HDL(mg/dl)} = \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} * \text{C Standard}$$

4. Measurement of serum low density lipoprotein (LDL)

Most of the circulating cholesterol is found in three major lipoprotein fractions: very low density lipoproteins (VLDL), LDL and HDL.

[Total chol] = [VLDL-chol] + [LDL-chol] + [HDL-chol]

LDL-cholesterol is calculated from measured values of total cholesterol, triglycerides and HDLcholesterol according to the relationship: [LDL-chol] = [total chol] - [HDL-chol] - [TG]/5 where [TG]/5 is an estimate of VLDL-cholesterol and all values are expressed in mg/dL.

5.Measurement of fasting blood glucose

Principle: Fasting glucose is measured by GOD-PAP enzymatic method with deproteinization on a fully automated Mindray BS-200 clinical chemistry analyzer. Glucose present in the serum is oxidized by the enzyme glucose oxidase (GOD) to gluconic acid with the liberation of hydrogen peroxide, which is converted to water and oxygen by the enzyme peroxidase (POD). 4-aminoantipyrine [4-AA], an oxygen acceptor, takes up the oxygen and together with phenol forms a red colored chromogen proportional to the concentration of glucose in the sample can be measured at 500nm(480 – 520 nm)

6. Hemoglobin A1c Measurement

whole blood is treated with lysing reagent and is then mixed with the anti-HbA1c antibody in a buffered reagent. The HbA1c in the sample forms a soluble complex with the anti-HbA1c. A poly-hapten reagent containing multiple HbA1c epitopes is then added to the sample, forming an insoluble complex with the excess free anti-HbA1c antibody. This antibody poly-hapten complex is then measured turbidimetrically at 340 nm. Two ratio calculation options are provided to the customer in the package insert. The first option reports the results in % HbA1c while the second option reports the results in SI units of mmol/mol total hemoglobin.

7. Creatinine Measurement

Creatinine under alkaline conditions reacts with picrate ions forming a reddish complex. The formation rate of the complex measured through the increase of absorbance in a prefixed interval of time is proportional to the concentration of Creatinine in the sample.

Creatinine +Alkaline picrate ⟶ Orange colored complex

$\frac{\Delta A\ sample - \Delta A\ blank}{\Delta A\ standard - \Delta A\ blank} \times$ 2(Standard conc.) = mg/dL of (Creatinine in Sample)

Conversion factor : mg/dL × 88.4 = µmol/L

8. Determination of Serum Urea

Principle:

Serum urea concentration is determined by enzymatic kinetic method on a fully automated Mindray BS-200 clinical chemistry analyzer. Urea is hydrolyzed by water and urease to produce ammonia and carbon dioxide. The ammonia produced is further acted with ketoglutarate and NADH in the presence of GLDH to reproduce glutamate and NAD+. There has been optimized so that the GLDH is the rate limiting enzyme. The decrease in absorbance due to the decrease of NADH concentration in unit time is proportional to the urea concentration.

Urea + H2O $\xrightarrow{\text{Urease}}$ $2NH_3 + CO_2$

$2NH4^+$ + 2 **α** ketoglutarate $\xrightarrow{\text{GLDH}}$ **γ** - Glutamate + $2NAD^+ + H_2O$

Calculations: C $= \frac{O.D1 - O.D2\ sample}{O.D1 - O.D2\ STD}$ x Standard concentration = mg/dL Urea

Conversion factor for BUN/Urea [mg/dl]

C (BUN) =0.47xC (Urea)

C (Urea) =2.14x C (BUN)

4.8. Data Quality Assurance

4.8.1. Pre-analytical

The Questionnaires were translated from English to Amharic version and the data collection after questioner was Pre tested on 10% diabetic patient in health center to see the validity and completeness. Then correction was taken and those participants who don't fulfill the criteria was excluded from the study. Training for data collectors were given by the investigator and the consistency of the data was checked.

For laboratory sample which was analyzed by Mindray BS-200 chemistry analyzer and Siemens Dimension 200, its quality was checked by previously documented SNNP, Regional Laboratory and EQA sample result feedback report in every three months. Concerning sample collection, transportation and processing the principal investigator were assemble blood sample collection materials. Principal investigator were strictly follow SOP to assure that sample was collected on serum separator tube and EDTA tube, labeling with participant identification number, allow the sample for minimum of 15 minutes to clot, transportation, and centrifuging sample for 5 minutes at 3000 revolution per minute.

4.8.2. Analytical

Standard quality control (normal and pathological) protocols were performed and passing prior to run the participants sample analyzing to assure the accuracy and functionality of the instrument. The participants sample was analyzed after both controls pass and interpreted using Westgard multi-rule algorithm.

4.8.3. Post-analytical

All necessary procedures and steps was followed based on the clinical chemistry SOP. Laboratory results were rechecked repeatedly for completeness and recorded carefully on the provided space by seeing its labeling and attached with its questionnaire and interpretations by choosing appropriate statistical tests.

4.9. Data analysis

All questionnaires were checked visually, coded and entered into EPI info and then exported to SPSS version 24.0 windows program for analysis. For controlling errors, the questionnaire was double entered; and also frequency checks were done. Descriptive statistics expressed by frequency, percentages and median with Inter quartile range. The associated factors were determined using bivariate and multivariate binary logistic regressions. Normality test has been done using Kosmoglov-Smirnov and Shapiro-Wilk test. Goodness of fit test had also computed for logistic regression using Hosmer and Lemeshow test.

Strength of association between dependent and independent variables was assessed using crude odds ratio (COR) and adjusted odds ratio (AOR) with confidence Interval (CI) of 95%. Variables

that had a value of P ≤0.25 on bivariate analysis were directly forward to be analyzed by multivariate analysis then having P-values < 0.05 is considered as statistically significant. The result is presented by using tables, chart and texts.

4.10. Ethical considerations

The study was approved by Addis Ababa University College of health sciences department of medical laboratory sciences Ethical review committee. Permission letter was written from Addis Ababa University to Wolaita Sodo University teaching and referral hospital managing director. For reasons of privacy, all data were kept confidential. Anonymity of result records was maintained by using client registration number and unique code numbers used by service providers at Wolaita Sodo University teaching and referral hospital Laboratory.

4.11. Operational definitions

Adherence to medication: if the patients took all his/ her anti-diabetic medication in the last seven days.

Alcohol consumption- if reported consumption of alcohol twelve-month prior to the survey.

Adherence to diet: If the respondents follow a recommended diet.

Abnormal lipid profile:-Serum lipid measurement in any one of the four lipid parameters, TC ≥ 200 mg/dl, HDL < 40 mg/dl, LDL≥ 130 mg/dl, TG ≥ 150 mg/dl.

Fasting blood sugar: blood glucose measured from venous blood after at least 8 h of overnight fasting.

Good glycemic control: means HbA1c level ≤7%.

Poor glycemic control: means HbA1c level >7%.

4.12. Dissemination of the result

The finding of this study will be submitted and presented to Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Sciences, as partial fulfillment of the requirement of master's degree in clinical chemistry, and the result was shared with Wolaita Zone Health Office and WSU Health sciences and medicine. The manuscript of the research will be submitted for publication on peer reviewed journals.

5. Result

5.1 Socio-demographic characteristics of study participants

Among total of 140 participants, 134 were involved in the study with a response rate of 96%. Three study participants were excluded from the study due to insufficient blood samples for laboratory analysis, two hemolysed sample and one disagreed to participate in this study because of the fear of vein puncture in the blood sample collection.

Most of the patients, 72 (53.7%) were males with male to female ratio 1.16. The age of study participants ranged from 25 years to 80 years with an overall mean +/-SD age of 48.4±10.6 years. Half of patients , 69 (51.5%) were aged between 41-55. The majority (71.6%) were protestant and half of the patients, 69 (51.5%) were illiterate, 132 (98.5%) were married, 68 (50.8%) were Others, as show in table 1.

Table 1. Socio-demographic status of study participants attending WSUTRH, SNNPR, Ethiopia from February 2021 to April 2021

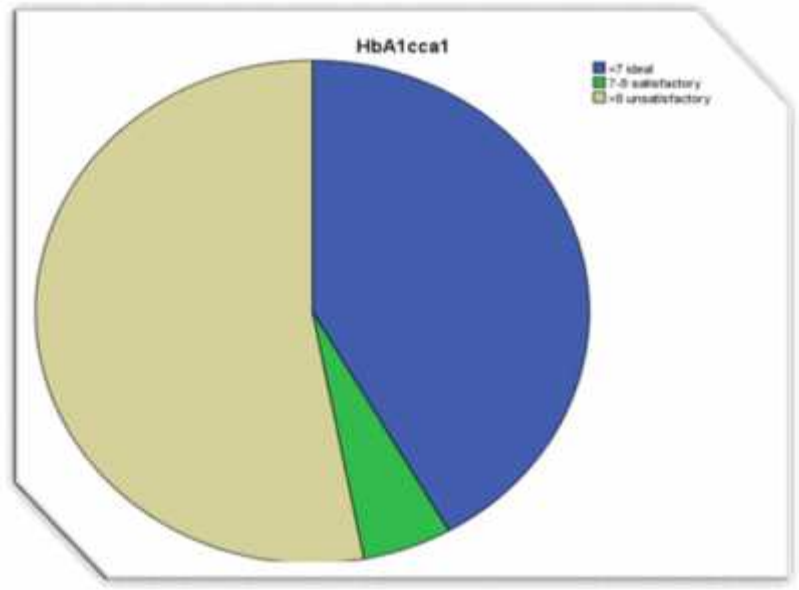
Variables N(134)	Categories	Number (%)
Sex	Male	72 (53.7%)
	Female	62 (46.3%)
Age	25-40	35 (26.1%)
	41-55	69 (51.5%)
	≥56	30 (22.4%)
Marital Status	Married	132 (98.5%)
	Unmarried	2 (1.5%)
Religion	Orthodox	29 (21.6%)
	Protestant	96 (71.6%)
	Muslim	9 (6.7%)
Occupational Status	Government	26 (19.4%)
	Merchant	15 (11.2%)
	Farmer	25 (18.7%)

	Other	68 (50.8%)
Residence	Rural	54 (40.3%)
	Urban	80 (59.7%)
Educational Status	Illiterate	69 (51.5%)
	Primary	15 (11.2%)
	Secondary	25 (18.7%)
	diploma and above	25(18.7%)

5.2 Level of HbA1c

Our study participants had a mean HBA1C of 7.9% (SD±1.6; median 7.6%). Based on HbA1c values the pattern of glycemic control among diabetic patients was determined, and 56 (41.8%) patients had good/Ideal glycemic control i.e. HbA1c <7 %, and 7 (5.2%) had satisfactory glycemic control (HbA1c between 7-8 %), while majority of patients 71 (53%) had unsatisfactory glycemic control (HbA1c value was more than 8 %). (Figure 1).

Of the 134 diabetics, 72 (53.7) were male and 62 (47.3) were female subjects. The percentage of females with poor glycemic control (42/62, 68 %) was higher compared with percentage of males with poor glycemic control (36/72, 50 %).



HbA1c < 7% is good glycemic control. HbA1c >7% poor glycemic control which includes satisfactory and unsatisfactory glycemic control

Figure 1 Degree of glycemic control among patients with diabetes

5.3 Levels of Total Cholesterol, LDL-c, HDL-c and Triglycerides

Of the 134 diabetics study participants , 11 (8.2%) had abnormal total cholesterol levels,(above 200 mg/dL.), 76(56.7%) diabetic patients showed abnormally high TG levels.
15(11.2%) diabetic patients had an abnormal LDL-c level (over 100 mg /dL For HDL-c levels, most 95 (70.9 %) of patients had normal HDL-c level. Whereas the remaining diabetic patients , 39 (29.1%) had abnormally low levels of serum HDL-c., as shown in table 2

Table 2 Number of diabetic patients with normal and abnormal serum levels of lipid profile at WSUTRH, SNNPR, Ethiopia from February 2021 to April 2021

Parameter	Serum level	N (%)
Total Cholesterol	Normal (≤200)	123 (91.8%)
	Elevated (>200)	11 (8.2%)
HDL-Cholesterol	Normal (≥35)	95 (70.9%)
	Low (<35)	39 (29.1%)
LDL-Cholesterol	Normal (≤100)	119 (88.8%)
	Elevated (>100)	15 (11.2%)
Triglyceride	Normal (≤150)	58 (43.3%)
	Elevated (>150)	76 (56.7%)

5.4 Anthropometric measurement and Clinical features in diabetic patients

Generally the study population had mean BMI value of 23.7 (SD ± 3.2 Kg/m^2). This shows that the study population had normal weight. When classified into the specific categories most of 93 (69.4%) participants were within the normal ranges of BMI (18.5 to 25 kg/m2), 36(26.9%) of the study population had BMI above 25 kg/m2 and were classified as either overweight or obese.

On the other hand, blood Pressure measurements of the study participants were measured, and had a mean value of 129/74 mm Hg. When classifying the individual BP readings 108 (80.6%) and 88 (65.7%) of the study participants were within the optimal target for diastolic and systolic BP readings (Normal: less than 120 **systolic** and 80 **diastolic**) respectively.

Meanwhile, on the co-morbidity analysis most common co-morbidities observed in our study participant type 2 diabetes mellitus patients were hypertension 55 (41%)

Highest number of the respondents 98 (73.1%) were living with diabetes for less than 7 years. Of the total participants, about 126 (94%) of were nonsmokers, 127 (94.8%) were non-alcohol drinkers, 90(67.2%) were physically inactive. DM patients were followed for an average of 5.9 (±3.9) years with a minimum of 1 year and a maximum of 20 years, shown in table 3).

Table 3 Clinical characteristics and disease self-care activities of diabetic patients attending WSUTRH, SNNPR, Ethiopia from February 2021 to April 2021

Variables N(134)	Categories	Number (%)
BMI	<18.5 or underweight	5 (3.7%)
	18.5-24.9 or normal weight	93 (69.4%)
	25-29.9 or overweight	31 (23.1%)
	>30 or obese	5 (3.7%)
Waist Circumference	>102 cm for M >88 for F	74 (55.2%)
	≤102 cm for M ≤88 for F	60 (44.8%)
Dose of metformin	0.5g	33 (24.6%)
	1g	61 (45.5%)
	2g	40 (29.9%)
Comorbidity	Yes	57 (42.5%)
	No	77 (57.5%)
Smoker	Yes	8 (6%)
	No	126 (94%)
Alcohol	Yes	7 (5.2%)
	No	127 (94.8%)
Duration of diabetes	≤7 years	98 (73.1%)
	>7	36 (26.9%)
Systolic blood pressure	≤120mmHg	88 (65.7%)
	>120mmHg	46 (34.3%)
Diastolic blood pressure	≤80 mmHg	108 (80.6%)
	>80 mmHg	26 (19.4%)

5.5 Comparison of laboratory test parameters among study groups

In the association of various biochemical parameters with glycemic control of our study participants, total cholesterol, Triglycerides, LDL-c, HDL-c and Creatinine indices showed no statistically significant differences between good and poor glycemic control cases. Most of the diabetic patients with poor diabetic controls (78/134) had blood cholesterol level of 142±37 mg/ml. whereas the remaining diabetic patients (56/134) with good diabetic control have blood cholesterol level of 136±28. The mean triglyceride, LDL and HDL of type 2 diabetic patients (134) were (167±53, 65±25 and 41±8), respectively. Most of the diabetic patients with poor controlled diabetes showed high level of TG and normal LDL, HDL (170±52, 68±27 and 40.9 ± 8.9) respectively. Whereas, the remaining diabetic patients with good controlled diabetes showed high levels of TG and normal levels of LDL and HDL (164± 54, 62±23 and 41±7) respectively. Most of the diabetic patients with poor diabetic controls (78/134) had fasting blood sugar level of 209 ±68. Whereas, the remaining diabetic patients (56/134) with good diabetic control have blood fasting blood sugar level 184 ±64. Poorly controlled diabetic patients had significantly elevated level of FBS (p=0.031) as compared with the good controlled diabetes.

, as shown in table 4.

Table 4 Mean values of various biochemical parameters of diabetic patients studied

Parameters	All patients	Glycemic Control		P-value
	N (134)	Well controlled diabetes N (56)	Poorly controlled diabetes N (78)	
FBS	198.90 ±68.03	184.00 ± 64.477	209.59 ± 68.899	0.031
T.Cholestrol	139.99 ± 34.149	136.12 ± 28.87	142.76 ± 37.417	0.269
HDLcholesterol	41.00 ± 8.230	41.13 ± 7.178	40.91 ± 8.953	0.882
LDLcholesterol	65.66 ± 25.596	62.39 ± 23.094	68.01 ± 27.154	0.211
Triglyceride	167.91 ± 53.304	164.11 ± 54.868	170.64 ± 52.338	0.486
Creatinine	.8962 ± .30055	.9252 ± .27316	.8754 ± .31886	0.346

(P-value refers to comparison of values for poorly controlled with controlled diabetics Units in Mg/dl

5.6 Effects of metformin in some biochemical based on dosage

Levels of serum lipid were compared between dosage-based subgroups using one-way analysis of variance. As shown in Table 5, increasing the dosage of metformin had significant effect on lipid-lowering efficacy. Meanwhile, improvement in HDL and LDL was similar between participants receiving 500 mg, 1,000 mg, and 2,000 mg of metformin per day. Two subgroups had comparable mean HbA1c, while those receiving 2,000 mg of metformin per day harbored higher fasting blood sugar.

Table 5 Comparison of clinical effect of metformin between dose-based subgroups.

Parameters	Metformin 500 mg/day n=33	Metformin 1,000 mg/day n=61	Metformin 2,000 mg/day n=40
FBS,mg/dl	190.3±68.9	197.4±66.2	208±70.7
T.Cholestrol,mg/dl	145.7±31.9	141.9±35.8	132.3±32.7
HDLcholesterol,mg/dl	42.1±8.0	41.4±8.4	39.5±8.1
LDLcholesterol,mg/dl	65.9±23.4	66.7±29.5	63.8±21.1
Triglyceride,mg/dl	183.1±58.1	168.1±41.1	155±62.8
HbA1c, %	6.3±0.6	8.1±0.9	8.9±1.9

5.7 Factors associated with poor glycemic control in diabetic patients

In the bivariate binary logistic regression analysis statistically significant values of study participants with poor glycemic control had seen in age, sex, occupational status, and education, T.Cholestrol, Triglyceride, HDL-C, Dose of Metformin and Comorbidity values.

.

However in multivariate binary logistic regression only four variables had significant association with poor glycemic control; including age, triglyceride, comorbidity and dose of metformin.

As shown in Table 5.6 for all the variables cross tabulated with HBA1C, in the multivariate analysis, Age (OR=8.87 P=0.005), triglyceride (OR=0.27 P=0.031), dose of metformin (OR=0.005 P=0.000) and comorbidity (OR=0.21 P=0.018) had significant association with poor glycemic controls.

Table 6 Bivariate and Multivariable Analysis of Factors Associated with DM Among patients attending WSUTRH, SNNPR, Ethiopia from February 2021 to April 2021

Variables		Glycemic Control		COR(95% CI)	P-Value	AOR(95% CI)	P-value
		Good	Poor				
Sex	Male	36	36	.47 (0.23-0.96)	0.039	2.557(.507-12.887)	.255
	Female	20	42	1		1	
Age	25-40	12	23	2.87(1.05-7.89)	0.040	3.754(.647-21.777)	.140
	41-55	26	43	2.48(1.03-5.97)	0.043	8.871(1.923-40.92)	.005
	>55	18	12	1		1	
Occupation	governm ent	9	6	.40(.158-1.028)	0.057	.243(.033-1.759)	.165
	Merchant	15	11	.36(.115-1.166)	0.089	.964(.078-11.842)	.977
	Farmer	9	16	.97(.371-2.575)	0.964	1.698(.072-39.372)	.741
	Other	23	45	1		1	
Educational status	Illiterate	16	9	2.16(.854-5.495)	0.103	4.874(.784-29.176)	.090
	Primary	13	12	2.97(.744-11.93)	0.123	4.989(.451-55.159)	.190

	secondary	23	46	.609(.196-1.891)	0.391	1.515(.246-9.333)	.654
	diploma	4	11	1		1	
T.Cholestrol	≤200	54	69	0.284(0.059-1.36)	0.117	.041(.002-1.010)	.051
	>200	2	9	1		1	
Triglyceride	≤150	29	29	0.551(0.274-1.16)	0.094	.271(.082-.890)	.031
	>150	27	49	1		1	
HDL-C	≥35	44	51	0.515(0.234-1.13)	0.100	.400(.116-1.381)	.147
	<35	12	27	1		1	
Dose of Metformin	0.5g	31	2	0.022(0.004-0.16)	0.000	.005(.000-.047)	.000
	1g	10	30	1.022(0.406-2.57)	0.963	.485(.100-2.353)	.369
	2g	15	46	1		1	
Comorbidity	Yes	30	27	.459(.227-.926)	0.031	.210(.058-1.279)	.018
	No	26	51	1		1	

Good-HbA1c level ≤7%, Poor-HbA1c level>7%

6. Discussion

Glycemic control may be a phrase given for the extent of blood sugar in polygenic disease patient, and smart glycemic management avoids the severity of complications and will increase psychological feature functioning. Glycemic management are often evaluated by mensuration the haemoprotein A1c (HbA1c) that notifies the typical glucose level within the past 2-3 months.

Estimation of Glycated hemoglobin (HbA1c) is currently all around acknowledged as the most dependable indicator of long haul glycemic control, since it precisely mirrors a person's blood glucose levels over the former 2-3 months. Appraisal of the degree of diabetes control in a populace utilizing HbA1c is a decent marker of the nature of diabetes care accessible to the populace. Appraisal of the current glycemic status and the weight of diabetes related confusions are in this way significant to assign local area and wellbeing assets in any country and plan mediations to deliver the issues identified with poor glycemic control(8)**.**

Results of this study showed that nearly two third of patients with T2DM had poor glycemic control. This result was comparable to those obtained in an earlier study that reported 48.7% (41) and 50% (22).

However, other studies reported higher proportion of patients with poor in glycemic control Malaysia (77% had HbA1c ≥6.5%) (44), India (78.6% had HbA1c ≥7%) (8)**,** Saudi Arabia (78% had >7%) (45)**,** Tanzania (69.7% had FBG of ≥7.2 mmol/L) (7) and southwest Ethiopia (81.7% had FBS ≥126 mg/dL) (46)**.** This could be explained that there may be a great effort to improve glycemic control and treatment outcomes among diabetic patients at the study area. The current finding was higher than from developed countries such as 12.9 % in United States and ADA Guidelines report that 26.3%(38)(47). This variation could be due to knowledge difference of respondents between developing and developed countries, absence of uniform guidelines in assessing glycemic control for physicians to set the score cutoff, and the presence health insurance and the difference in health insurance coverage and access to primary care(47)(48)(49).

Of the total 134 patients, 58.2% had a mean HbA1c level 7.9% resulting in poor glycemic control. This result was in line with Ghazanfari et al Iran conducting using HbA1C as

measurement of glycemic control(50) However, our finding was lower than other similar study conducted in India and Ethiopia(46)(51)**.**

The current study showed that age, triglyceride, dose of metformin and comorbidity were associated with poor glycemic control.

The mean age of patients in our study was 48.4 (SD = 10.6) years which was a bit higher than other studies(46)(50)**.** In this study, a significant association was found between glycemic control and age which was consistent with previous study findings(7)(41)**.** Most patients with poor glycemic control belonged to the age categories 41-55 years, which was similar to the studies reported by Woldu etal (22)**.**

However, a study done in MGM medical college, India, showed that age was not statistically significantly associated with glycemic control (35). The noticed variety of relationship among age and poor glycemic control could be clarified by the distinctions in populace pyramids and circulation old enough in various examinations. More youthful people are bound to have more obstructions to self-administration practices like solid low-fat eating routine, glucose testing, and consistence with their eating regimen and drugs.

Ongoing investigations showed that poor glycemic control may prompt decreased renal capacity, endothelial brokenness, nephropathy and dyslipidemia. Subsequently, diabetic patients ought to be encouraged to keep up great glycemic control to forestall the difficulties related with diabetic dyslipidemia. Anyway the glycemic control without help from anyone else may not be adequate to control diabetic dyslipidemia. Alongside glycemic control, diabetic subjects may likewise require direct lipid the board. Not many enemies of diabetic treatments like metformin have additionally demonstrated to be useful in keeping up better glycemic control and improving dyslipidemia (34).

Lipid abnormalities are common in patients with diabetes. Characteristically, they have elevated triglyceride levels, while HDL levels are low, and LDL levels are typically normal or elevated. In this study, dyslipidemia was associated with poor glycemic control, especially for triglycerides. The study found no significant association between duration of diabetes, LDL, HDL and blood pressure. This was consistent with (38).

Studies by Adham et al (35) and Benoit et al (36) revealed that factors related to better glycemic control were lower levels of total cholesterol, low-density lipoprotein cholesterol and triglycerides (5). The National Cholesterol Education Program (NCEP) also provides that lipid-lowering treatments are fundamental component of diabetes care (52).

In the current study, patients who had hyperlipidemia, have a 0.27 times low risk to develop poor glycemic control than patients with no hyperlipidemia. Other studies have also shown that there was a positive correlation between triglyceride and poor glycemic control(40)(53). Perhaps the high level of the triglyceride among patients with poor glycemic control was the result of the poor glycemic control rather than cause. It is difficult to differentiate the cause/effect relation between dyslipidemia and poor glycemic control by cross sectional study.

Metformin may counter the derangements in lipid metabolism in T2DM through several pathways. Through increasing insulin sensitivity, metformin reduces the rate of lipolysis, thereby slowing the conversion of free fatty acids to lipoprotein precursors in the liver. By reducing plasma glucose levels, metformin lowers the fraction of irreversibly Glycated LDL-C, which is removed less efficiently from the body. Metformin also improves dyslipidemia by inducing weight loss in people with impaired glucose metabolism (54).

As observed in the current study, metformin monotherapy lowered Triglyceride level, whereas serum HDL-C did not significantly improved. This lipid-modifying effect is concordant with existing evidence that diminished HDL-C require a longer therapeutic duration to counteract than lowering LDL-C(54). Moreover, metformin's effect on serum lipid appeared to be dosage-independent in this study, suggesting this medication may control dyslipidemia through indirect pathways.

Considering the expense and expected symptoms of lipid-bringing down medication like statins (55)**,** individuals with dyslipidemia who are ineligible for lipid-bringing down treatment may regardless profit by metformin treatment. Past examiners have additionally noticed a synergistic impact among metformin and statin (56)**,** which can additionally decrease cardiovascular occasions in danger people.

Patients who have one or more comorbidity have higher odds of having poor glycemic control. The study conducted in Mekelle town found that patient who had hypertension as one part of comorbidity were more likely to be poorly controlled (41). Similarly, a study done in India showed that the presence of diseases such as coronary heart disease, neuropathy, retinopathy, renal failure and neurological disorders was associated with poor control of diabetes(57).

The current examination showed that term of diabetes and BMI were not related with poor glycemic control. The absence of relationship between these variables and glycemic control in our examination is conversely with the discoveries done in Tanzania(7). Kamuhabwa and Charles have shown the more extended term of the diabetes was related with poor glycemic control(7). The conceivable clarification of the relationship between the more drawn out term of diabetes and the poor glycemic control is the weariness of the pancreas to deliver more insulin. The distinction in the outcomes between our discoveries and the past ones could be clarify by the distinction in the socio-segment and ethnic qualities.

Employment and Education were not associated with poor glycemic control in the current study. This goes with the previous report from Tanzania (7) and Sudan(53) where education was not associated with glycemic control. Education could be an important tool to raise patient awareness and have a positive impact on glycemic control.

7. Strength and Limitation of the study

7.1 Strength

- HbA1c test was used which increases quality of test result.
- Data and sample collection was done by well-experienced nurses and laboratory technologists with the strict following of SOPs.
- Analyses of laboratory tests were done with fully automated clinical chemistry instruments.

7.2 Limitation

- The sample size was small making it difficult to generalize the findings.
- This study was limited by cross-sectional study, which could not provide a well-established association between glycemic control and its potential predictors, unlike a longitudinal design.
- It was done only among type 2 diabetes patients who were on follow up at outpatient clinic which may not be representative of the overall type 2 diabetes population.
- Other factors (hemoglobinopathies, change in erythrocyte life span) that may have an influence on HbA1c were not investigated.

8. Conclusion and Recommendation

8.1 Conclusion

To summarize, nearly two-third (58.2%) of diabetic patients attending at Wolaita Sodo University Teaching Referral Hospital in Southern Ethiopia had poor glycemic control. The variables found to influence the outcome of glycemic control in the present study were age, comorbidity, Triglyceride and dose of metformin.

In people with T2DM, metformin therapy significantly reduced both serum T.Cholestrol and TG, without concomitant lipid-lowering medications. Moreover, the lipid-modifying effect of metformin appeared to be dosage dependent. Overall, metformin is a safe and efficacious approach to alleviate dyslipidemia in people with T2DM.

8.2 Recommendation

- Measurement of glycated hemoglobin (HBA1c) would show the rate of glycemic control over a 3-month period, which is the gold standard to determine patient's "glycemic level" so for all type 2 DM patients HBA1c should be ordered during follow up.
- By facilitating investigation and case management process it is better to curtail patient's life expectancy.

9. References

1. Guangcui Xu YZ. Type 2 Diabetes Mellitus- Disease, Diagnosis and Treatment. *J Diabetes Metab*. 2015;06(05).

2. Zheng Y, Ley SH, Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *Nat Rev Endocrinol* [Internet]. 2018;14(2):88–98. Available from: http://dx.doi.org/10.1038/nrendo.2017.151

3. Hayashino Y, Izumi K, Okamura S, Nishimura R, Origasa H, Tajima N. Duration of diabetes and types of diabetes therapy in Japanese patients with type 2 diabetes: The Japan Diabetes Complication and its Prevention prospective study 3 (JDCP study 3). *J Diabetes Investig*. 2017;8(2):243–9.

4. Ireland S, Endacott R, Cameron P, Fitzgerald M, Paul E. Prospective Observational Study ಜ . Resuscitation [Internet]. 2010;(Ukpds 35):18–23. Available from: http://dx.doi.org/10.1016/j.resuscitation.2010.10.016

5. Haghighatpanah M, Sasan A, Nejad M, Haghighatpanah M, Thunga G, Mallayasamy S. Article history: Factors that Correlate with Poor Glycemic Control in Type 2 Diabetes Mellitus Patients with Complications Osong Public Health and Research Perspectives-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/). Public Heal Res Perspect. 2018;9(4):167–74.

6. Juarez DT, Sentell T, Tokumaru S, Goo R, Davis JW, Mau MM. Factors associated with poor glycemic control or wide glycemic variability among diabetes patients in Hawaii, 2006-2009. *Prev Chronic Dis*. 2012;9(9):1–10.

7. Kamuhabwa AR, Charles E. Predictors of poor glycemic control in type 2 diabetic patients attending public hospitals in Dar es Salaam. *Drug Healthc Patient Saf*. 2014;6:155–65.

8. A Kakade A, R Mohanty I, Rai S. Assessment of factors associated with poor glycemic control among patients with Type II Diabetes mellitus. *Integr Obes Diabetes*. 2018;4(3):1–6.

9. Kim YS, Cho BL, Kim WS, Kim SH, Jung IH, Sin WY, et al. Frequency and severity of hypoglycemia in type 2 diabetes mellitus patients treated with a sulfonylurea-based regimen at university-affiliated hospitals in Korea: The naturalistic evaluation of hypoglycemic events in diabetic subjects study. *Korean J Fam Med.* 2019;40(4):212–9.

10. Bristol-Myers Squibb. U.S. FDA. (metformin hydrochloride) Tablets Mechanism of Action. 2009;1–35.

11. Al-Eitan LN, Almomani BA, Nassar AM, Elsaqa BZ, Saadeh NA. Metformin pharmacogenetics: Effects of SLC22A1, SLC22A2, and SLC22A3 polymorphisms on glycemic control and HBA1c levels. *J Pers Med*. 2019;9(1):1–11.

12. Garber AJ, Donovan DS, Dandona P, Bruce S, Park JS. Efficacy of glyburide/metformin tablets compared with initial monotherapy in type 2 diabetes. *J Clin Endocrinol Metab*.

2003;88(8):3598–604.

13. Papanas N, Maltezos E. Metformin: A Review of Its Use in the Treatment of Type 2 Diabetes. *Clin Med Ther*. 2009;1:CMT.S1085.

14. Nanditha A, Ma RCW, Ramachandran A, Snehalatha C, Chan JCN, Chia KS, et al. Diabetes in Asia and the pacific: Implications for the global epidemic. *Diabetes Care*. 2016;39(3):472–85.

15. Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW, et al. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Res Clin Pract* [Internet]. 2018;138:271–81. Available from: https://doi.org/10.1016/j.diabres.2018.02.023

16. Facts F, Diabetes ON. centers for disease control and prevention. National Diabetes Fact Sheet,. Nj [Internet]. 2011;07470:973–696. Available from: http://sunshinepando.com/wp-content/uploads/2015/01/national-diabetes-fact-sheet-2011-Sunshine-Prosthetics-and-Orthotics-Wayne-NJ.pdf

17. Liu X, Yu C, Wang Y, Bi Y, Liu Y, Zhang ZJ. Trends in the incidence and mortality of diabetes in china from 1990 to 2017: A joinpoint and age-period-cohort analysis. *Int J Environ Res Public Health.* 2019;16(1).

18. Mobula LM, Sarfo FS, Carson KA, Burnham G, Arthur L, Ansong D, et al. Predictors of glycemic control in type-2 diabetes mellitus: Evidence from a multicenter study in Ghana. Transl *Metab Syndr Res* [Internet]. 2018;1:1–8. Available from: https://doi.org/10.1016/j.tmsr.2018.09.001

19. R A, JI D, EAM G, T B, D B, AP K. Diabetes in sub-Saharan Africa: from clinical care to health policy. *Yearb Paediatr Endocrinol*. 2018;5(August):622–67.

20. Yigazu DM, Desse TA. Glycemic control and associated factors among type 2 diabetic patients at Shanan Gibe Hospital, Southwest Ethiopia. *BMC Res Notes*. 2017;10(1):1–6.

21. Fekadu G, Bula K, Bayisa G, Turi E, Tolossa T, Kasaye HK. Challenges and factors associated with poor glycemic control among type 2 diabetes mellitus patients at nekemte referral hospital, Western Ethiopia. *J Multidiscip Healthc*. 2019;12:963–74.

22. Alebachew Woldu M, Diriba Wami C. Factors Associated with Poor Glycemic Control among Patients with Type 2 Diabetes Mellitus in Ambo Hospital, Ambo; Ethiopia. *Endocrinol Metab Syndr*. 2014;03(04):2–7.

23. Desse TA, Eshetie TC, Gudina EK. Predictors and treatment outcome of hyperglycemic emergencies at Jimma University Specialized Hospital , southwest. *BMC Res Notes.* 2015;1–8.

24. Kassahun T, Eshetie T, Gesesew H. Factors associated with glycemic control among adult patients with type 2 diabetes mellitus: A cross-sectional survey in Ethiopia Endocrine Disorders. *BMC Res Notes*. 2016;9(1):1–6.

25. Silva EFF, Ferreira CMM, De Pinho L. Risk factors and complications in type 2 diabetes outpatients. *Rev Assoc Med Bras*. 2017;63(7):621–7.

26. Wu Y, Ding Y, Tanaka Y, Zhang W. Risk factors contributing to type 2 diabetes and recent advances in the treatment and prevention. *Int J Med Sci.* 2014;11(11):1185–200.

27. Diabetes DOF. Diagnosis and classification of diabetes mellitus. *Diabetes Care.* 2009;32(SUPPL. 1).

28. Balaji R, Duraisamy R, Santhosh Kumar MP. Complications of diabetes mellitus: A review. *Drug Invent Today*. 2019;12(1):98–103.

29. Olokoba AB, Obateru OA, Olokoba LB. Type 2 Diabetes: a Review of Current Trends -. *Int J Curr Res Rev*. 2015;7(18):61–6.

30. Access O. Type 2 Diabetes - From Pathophysiology to Modern Management [Working Title]. Type 2 Diabetes - From Pathophysiol to Mod Manag [Working Title]. 2019;

31. Kahn SE, Cooper ME, Del Prato S. Pathophysiology and treatment of type 2 diabetes: Perspectives on the past, present, and future. Lancet [Internet]. 2014;383(9922):1068–83. Available from: http://dx.doi.org/10.1016/S0140-6736(13)62154-6

32. Lebovitz HE. Type 2 diabetes: An overview. Clin Chem. 1999;45(8 II):1339–45.

33. Lotfy M, Adeghate J, Kalasz H, Singh J, Adeghate E. Chronic Complications of Diabetes Mellitus: A Mini Review. *Curr Diabetes Rev*. 2016;13(1):3–10.

34. Kaithala C, Namburi HK, Bandaru SS, Bandaru SBS, Adla N, Puchchakayala G. Prevalence of dyslipidemia and its association with glycemic control in indian type 2 diabetes population. *Rom J Diabetes, Nutr Metab Dis*. 2016;23(3):277–83.

35. Khattab M, Khader YS, Al-Khawaldeh A, Ajlouni K. Factors associated with poor glycemic control among patients with Type 2 diabetes. *J Diabetes Complications* [Internet]. 2010;24(2):84–9. Available from: http://dx.doi.org/10.1016/j.jdiacomp.2008.12.008

36. Benoit SR, Fleming R, Philis-Tsimikas A, Ji M. Predictors of glycemic control among patients with Type 2 diabetes: A longitudinal study. *BMC Public Health*. 2005;5:90–5.

37. Bukhsh A, Khan TM, Nawaz MS, Ahmed HS, Chan KG, Goh BH. Association of diabetes knowledge with glycemic control and self-care practices among pakistani people with type 2 diabetes mellitus. *Diabetes, Metab Syndr Obes Targets Ther.* 2019;12:1409–17.

38. Nduati NJ. Factors Associated With Glycemic Control among Type 2 Diabetes Patients Attending Mathari National Teaching Hospital, Nairobi Kenya. *J Endocrinol Diabetes* . 2016;3(6):1–11.

39. Kibirige D, Akabwai GP, Kampiire L, Kiggundu DS, Lumu W. Frequency and predictors of suboptimal glycemic control in an african diabetic population. *Int J Gen Med*.

2017;10:33–8.

40. Omar SM, Musa IR, Osman OE, Adam I. Assessment of glycemic control in type 2 diabetes in the Eastern Sudan. *BMC Res Notes* [Internet]. 2018;11(1):1–5. Available from: https://doi.org/10.1186/s13104-018-3480-9

41. Eticha T, Mulu A, Gebretsadik H, Kahsay G, Ali D, Rajeshwar Y. Factors associated with poor glycemic control in type 2 diabetic patients investigated at Ayder referral hospital, Mekelle, Ethiopia *Ijppr. Human.* 2016;6(3):160–71.

42. Gamo Sagaro G. Patients' Satisfaction and Associated Factors Among Outpatient Department at Wolaita Sodo University Teaching Hospital, Southern Ethiopia: A Cross Sectional Study. *Sci J Clin Med.* 2015;4(5):109.

43. Anderson N, Tobin JN, Zazula T. National Cholesterol Education Program (Ncep). Encycl Heal Behav. 2012;

44. Ahmad NS, Islahudin F, Paraidathathu T. Factors associated with good glycemic control among patients with type 2 diabetes mellitus. *J Diabetes Investig.* 2014;5(5):563–9.

45. Al-Quwaidhi AJ, Pearce MS, Sobngwi E, Critchley JA, O'Flaherty M. Comparison of type 2 diabetes prevalence estimates in Saudi Arabia from a validated Markov model against the International Diabetes Federation and other modelling studies. *Diabetes Res Clin Pract* [Internet]. 2014;103(3):496–503. Available from: http://dx.doi.org/10.1016/j.diabres.2013.12.036

46. Angamo MT, Melese BH, Ayen WY. Determinants of Glycemic Control among Insulin Treated Diabetic Patients in Southwest Ethiopia: Hospital Based Cross Sectional Study. *PLoS One*. 2013;8(4).

47. Coates RJ, Yoon PW, Zaza S, Ogden L, Thacker SB. Rationale for Periodic Reporting on the Use of Selected Adult Clinical Preventive Service - United States. Morb Mortal Wkly Rep [Internet]. 2012;61 Suppl:3–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22695456

48. Islam SMS, Niessen LW, Seissler J, Ferrari U, Biswas T, Islam A, et al. Diabetes knowledge and glycemic control among patients with type 2 diabetes in Bangladesh. *Springerplus.* 2015;4(1).

49. Wee HL, Ho HK, Li SC. Public Awareness of Diabetes Mellitus in Singapore. *Singapore Med J*. 2002;43(3):128–34.

50. Ghazanfari Z, Niknami S, Ghofranipour F, Larijani B, Agha-Alinejad H, Montazeri A. Determinants of glycemic control in female diabetic patients: A study from Iran. Lipids *Health Dis*. 2010;9:12–4.

51. Harrabi I, Harbi F Al, Ghamdi S Al. Predictors of Glycemic Control among Patients with Type 2 Diabetes in Najran Armed Forces Hospital: A Pilot Study. *J Diabetes Mellit.* 2014;04(02):141–7.

52. Saydah SH, Fradkin J, Cowie CC. Poor Control of Risk Factors for. Jama. 2004;291(3):335–42.

53. Noor SK, Elmadhoun WM, Bushara SO, Almobarak AO, Salim RS, Forawi SA, et al. Glycaemic control in Sudanese individuals with type 2 diabetes: Population based study. *Diabetes Metab Syndr Clin Res Rev.* 2017;11:S147–51.

54. Lin SH, Cheng PC, Tu S Te, Hsu SR, Cheng YC, Liu YH. Effect of metformin monotherapy on serum lipid profile in statin-naïve individuals with newly diagnosed type 2 diabetes mellitus: A cohort study. *PeerJ*. 2018;2018(4):1–10.

55. Raymond C, Cho L, Rocco M, Hazen SL. New cholesterol guidelines: Worth the wait? *Cleve Clin J Med*. 2014;81(1):11–9.

56. Kashi Z, Mahrooz A, Kianmehr A, Alizadeh A. The role of metformin response in lipid metabolism in patients with recent-onset type 2 diabetes: HbA1c level as a criterion for designating patients as responders or nonresponders to metformin. *PLoS One*. 2016;11(3):1–10.

57. Sanal TS, Nair NS, Adhikari P. Factor Associated With poor Control of Type 2 Diabetes Mellitus: A Systematic Review and Meta Analysis. *J Diabetol* [Internet]. 2011;3(1):1–10. Available from: http://www.journalofdiabetology.org/

10. Annexes

Annex 1 (A): Information sheet (English Version)

Title of the Research Project: Assessment of glycemic control among Type 2 Diabetes Mellitus patients on metformin treatment in Wolaita Sodo University Teaching Referral Hospital, SNNPR, Ethiopia.

Principal Investigator: Eyouel Shimeles (BSc, MSc candidate)

Name of the Organization: Addis Ababa University College of Health Science, School of Allied Health Science Department of Medical Laboratory Science

Introduction

You are kindly invited to participate as a study subject in a research conducted by Addis Ababa University College of Health Science, Department of Medical Laboratory Masters student thesis. Your participation is voluntary. The research teams will include one principal investigator, two advisors from Addis Ababa University. Please take as much time as you need to read or listen to the information sheet.

Purpose of the Research Project

We are asking you to take part in this study because we will try to assess glycemic control among Type 2 Diabetes Mellitus patients on metformin treatment and recommending tangible solutions to minimize complication of diabetes.

Procedures and the expected participation

If you are willing to participate, you need to understand the purpose of the study and give your consent. Not only this but also a specimen collected from you will be used for the research purpose, and the results of your sample will be exposed to some concerned professional staff as it is needed. The required clinical sample will be collected by residents of the clinical chemistry department. Then, you are requested to give your consent to the sample collector.

After consent, a sample will be taken from capillary and venous puncture. Moreover, there will be a face-to-face interview for additional questions.

Potential risks and Discomforts

During the collection of specimens from you, appropriate precaution will be taken and all samples will be collected by trained health professionals. If anything happened, appropriate medical care will be provided to you.

Confidentiality

We respect your privacy and confidentiality. Any information that identifies you will not be shared with anyone else outside the study team. The information we will collect from you as part of the study will be kept in a locked file cabinet, or be protected by a password on the computer only accessible to personnel involved in the study. There is no sensitive issue that you will be asked related with your social desirability but any information that is obtained in connection with this study and that can be identified with you will remain confidential.

Potential benefits to subjects and/or to the society

You will not receive any payment for your participation in this research study as compensation. However, based on the diagnosis result you will be treated in view of that. Hence, you are indirectly benefiting other patients and the society in this respect.

Participation and Withdrawal from the Study

The participation is voluntary and you have the right not to participate in this study. You may withdraw at any time and place without consequences of any kind. You may also reject to give any sample. You can ask any questions regarding to this study and you have a right to get a laboratory diagnosis result free.

Contact information: If you have any questions about this study you can contact the following principal investigators and advisors for further information.

Eyouel Shimeles Phone: +251982434670 E-mail: eyuyeshime@gmail.com

Dr. Mistire Wolde Phone: +251911699710 Email: mistire08@gmail.com

Mekdes Alem Phone: +251913601036 Email:mk.alem12@gmail.com

Annex 1(B): Information sheet (Amharic version)

የተሳታፊዎች ፈቃድና መተማመኛ ቅፅ

በአዲስአበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ ሳይንስ ት/ክፍል በማስተርስ ድግሪ ተማሪ የመመረቂያ ጥናት ላይ እዲሳተፉ ተጋብዘዋል፡፡ እባክዎ በዚህ ጥናት ለመሳተፍ ከመስማማትዎ በፊት ከዚህ ቀጥሎ የሚገኘዉን ምንባብ በጥሞና ያንብቡና ግልጽ ያልሆነልዎትን ማንኛዉም ሃሳብ ይጠይቁ፡፡

መግቢያ

የጥናቱ ርዕስ “Assessment of glycemic control among Type 2 Diabetes Mellitus patients on metformin treatment in Wolaita Sodo University Teaching Referral Hospital, SNNPR, Ethiopia.”

የእርስዎ በዚህ ጥናት ላይ የሚኖርዎት ተሳትፎ ሙሉ በሙሉ በበጎ ፈቃደኝነት ላይ የተመሰረተ ነዉ፡፡ በዚህ ጥናት ዉስጥ ላለመሳተፍ ወይም ለመሳተፍ ከወሰኑ በኃላ ለማቋረጥ የሚወስኑ ቢሆንም እንኩዋ በዚህ ሆስፒታል የሚሰጠዉ ማንኛዉም አገልግሎት አይቋረጥም፡፡በጥናቱ ለመሳተፍ የሚስማሙ ከሆነ የስምምነት ቅጹ ላይ በጹሁፍ ወይም በጣት ፊርማ ማስቀመጥ ይጠበቅዎታል፡፡

የጥናቱ ተሳታፊ ለመሆን የሚጠበቅበዎት ምንድን ነው?

በዚህ ጥናት ለመሳተፍ የሚስማሙ ከሆነ ናሙናዎ ለጥናቱ እንዲሚዉል መስማማት ይጠበቅብዎታል፡፡ ከተወሰደዉ ናሙና ላይ የሚገኙ መረጃዎች ከዚህ ሆስፒታል ዉጭ ለሚገኙና ለስራዉ አግባብነት ላላቸዉ ሰዎች ቢነገር የማይቃወሙ መሆኑን መስማማት ይጠበቅብዎታል፡፡ይሁን እንጅ ይህ አይነቱ መረጃ የርስዎን ማንነት የሚገልጡ መረጃዎችን ማለትም ስም፣አድራሻና የስልክ ቁጥር የመሳሰሉትን መረጃዎቸን አይጨምርም፡፡ይልቁንም ለዚህ አገልግሎት ብቻ የሚዉል እርስዎን ለማወቅ የሚያስችል መለያ ቁጥር ጥቅም ላይ እንዲዉል ይደረጋል፡፡ በተጨማሪም ስለርስዎ አጠቃላይ የጤና ሁኔታ ለሚቀርቡ አንዳንድ ተጨማሪ ጥያቄዎች መልስ መስጠት ይኖርብዎትአል፡፡

በዚህ ጥናት መሳተፍ የሚያስከትላቸዉ ችግሮች ምንድን ናቸዉ?

ናሙና በሚሰበሰብበት ወቅት ምንም አይነት የከፋ ችግር አያጋጥምዎትም፡፡ሆኖምግን ናሙናዉን ለመሰብሰብ ልምድ ያለዉ ባለሙያ ስለሚመደብና አስፈላጊዉ የጥንቃቄ እርምጃ ስለሚወሰድ የህመም ስሜት አይኖርም፡፡

የህክምና መረጃ በሚስጥር ተጠብቆ መቆየት የሚችለዉ እንዴትነዉ?

ስለራስዎ የሰጡት ማንኛዉም መረጃና ከተወሰደዉ ናሙና ላይ የተገኘዉ የላቦራቶሪ ዉጤት የሚዉለዉ ለጥናቱ አላማ ብቻ ነዉ፡፡ይህን ማህደር ሊያገኙ የሚችሉት የተወሰኑ የጥናቱ ተባባሪ ሰዎች ብቻ ናቸዉ፡፡ከዚያም በላይ ስለእርስዎ ያለውን ማንኛውንም መረጃ የተለየ የይለፍ ቃል ባለው የኮምፒውተር የመረጃ ማህደር ውስጥ እንዲቀመጥ ይደረጋል፡፡

በዚህ ጥናት መሳተፍ የሚያስገኛቸው ጥቅሞች ምንድን ናቸዉ ?

ይህ ጥናት የማስተርስ ድግሪ መመረቂያ እንደመሆኑ መጠን በዚህ ጥናት በመካፈልዎ በግንዘብ የሚያገኙት ጥቅም ባይኖርም ከጥናቱ በሚገኝው ውጤት ግን ተጠቃሚነዎት፡፡የእርሶዎ ተሳትፎ የእርስዎንና የወገንዎትን የደም ስኳር፣የኩላሊትህመም እና የኮሌስትሮል መጠን ለማወቅና ለማከታተል ከፍተኛ ጥቅም ይኖረዋል፡፡

በዚህ ጥናት ተሳታፊ የመሆንዎ መብቶች ምንድን ናቸዉ ?

በዚህ ጥናት መሳተፍ ሙሉ በሙሉ በእርስዎ ፈቃደኝነት የተመሰረተ በመሆኑ በማንኛውም ሰዓትና ቦታ የማቋረጥ ሙሉ መብት የተጠበቀ ከመሆኑም በላይ እራስዎን ከጥናቱ በማግለልዎ ምክንያት የሚቀርብዎት ምንም አይነት የሆስፒታል አገልግሎት አይኖርም፡፡ከዚህም በተጨማሪ ጥናቱን በተመለከተ ማንኛውንም አይነት ጥያቄ የመጠየቅና ገለጻ የማግኘት መብት አለብዎት፡፡ የላብራቶሪ ምርመራ ውጤቱንም በነጻ ማግኘት ይችላሉ፡፡ነገር ግን እርስዎ በሚሰጡን መረጃ የችግሩን ስፋት ለመከላከል እና ለመቆጣጠር ጠቃሚ ስለሆነ ለሚቀርብልዎት ጥያቄ ቀጥተኛ መልስ ይሰጡን ዘንድ በታላቅ አክብሮት እንጠይቃለን፡፡

ጥያቄ ካለኝ ወይም ችግር ቢያጋጥመኝ ምን ማድረግ ይገባል?

ይህንን ጥናት በተመለከተ ወይም ከዚህ ጥናት ጋር በተዛመደ መልኩ ስለሚያጋጥሙ ድንገተኛ አደጋዎች ወይም ጥያቄ ካለዎት በሚመለከተው አድራሻ ይጠቀሙ፡

እዩኤል ሽመልስ

ሞባይል፡ +251-982-434-670

ኢ-ሜል፡eyuyeshime@gmail.com

ዶ/ር፡ሚስጥረ ወልዴ

ሞባይል፡+251-911-699-710

ኢ-ሜል፡mistire08@gmail.com

መቅደስ አለም

ሞባይል፡ +251-913-601-036

ኢ-ሜል፡mk.alem12@gmail.com

Annex 2 (A): Informed consent form (English version)

Unique code:_____ Date:_____

I had been informed that the objective of this study is Assessment of glycemic control among Type 2 Diabetes Mellitus patients on metformin treatment in Wolaita Sodo University Teaching Referral Hospital, SNNPR, Ethiopia. The results of this study have an importance to treat me and other patients, and to be used as an input for the future development of strategies or guidelines for diagnosing of diabetes mellitus in Ethiopia. I had been also informed about the confidentiality of this study. The principal investigator requested me to participate in the study that would require my willingness to provide the required data that include blood sample, and filling questionnaire. Therefore, with full understanding of the importance of the study, I agreed voluntarily to provide the requested samples and my benefit will be only from the free laboratory investigation result/s.

I_____ hereby give my consent for providing the requested information and specimens as the doctors find best for me.

Signature: _____ Date_____

Annex 2 (B): Informed consent form (Amharic version)

የተሳታፊዎች ስምምነት ሚጋገጫ

የሚስጥር ቁጥር ----------

እኔ ስሜ ከላይ የተጠቀሰው ተሳታፊ "Assessment of glycemic control among Type 2 Diabetes Mellitus patients on metformin treatment in Wolaita Sodo University Teaching Referral Hospital,SNNPR,Ethiopia."**ጥናት ላይ በቂ ገለጻ ተደርጎልኛል፡፡ለጥናቱም የደም ናሙና እንደሚያስፈልግ ተገልጸልኛል፡፡የጥናቱንም አላማዎችም ተረድቻለሁ፡፡**

በቃለ መጠይቁ ላይ የገለጽኳቸው መረጃዎች በሙሉ በሚስጥር የተጠበቁ እንደሚሆኑ ተነግሮኛል፡፡በጥናቱ ላይ ያለመሳተፍና ማንኛውንም መረጃ ያለመስጠት እንዲሁም በማንኛውም ጊዜ ከጥናቱ ራሴን የማግለል መብቴ የተጠበቀ እንደሆነ ተገልጸልኛል፡፡

ስለዚህ ለዚህ ጥናት መረጃና የስምምነት ቃሌን የሰጠሁት በአጠቃላይ ሁኔታውን በመረዳትና በፍጹም ፍቃደኝነት ነው፡በተጨማሪም ጥያቄ ለመጠየቅ ተፈቅዶልኝ ለማወቅ የፈለኩትን ያህል ማብራሪያ አግኝቻለሁ፡፡ የዚህ ጥናት ተሳታፊ በመሆኔ የማገኘው ጥቅም የሁሉንም ምርመራ ውጤት በነጻ ማግኘት እንደሆነ ተረድቻለሁ፡፡

በአጠቃላይ እኔ ከላይ በመተማመኛ ቅፅ የተጠቀሱትን ሁሉ በሚገባና በተረጋጋ መንፈስ አንብቤዋለሁኝ፡፡ስለዚህ በዚህጥ ናት ለመሳተፍ ፈቃደኛ መሆኔን በፊርማዬ አረጋግጣለሁ፡፡

ፊርማ---------- ቀን -------/---/----------

(የስምምነት ቅጹን ማንበብ ለማይችሉ ተሳታፊዎች)

የአማካሪነርስስም ---------- ፊርማ ----------ቀን----------

Annex 3 (A): Structured Questionnaire for diabetic Study participants (English Version)

Participant's identification code: _____ Date _____

SECTION-1: SOCIO-DEMOGRAPHIC FACTORS

S.No	Questions	Responses
1	Age	**Years**
2	Sex	1.Male 2.Female
3	Marital Status	1. Single 2. Married 3. Co-habiting 4. Separated
4	Religion	1. Orthodox 2. Protestant 3. Muslim 4. other, specify_____
5	Education Level	1. No formal education 2. Primary 3. Secondary 5. College or University
6	Residence	1.Urban 2.Rural
7	Occupation	1. Gov.t Employee 2.Merchant 3. Farmer 4. Daily laborer 5.Other

SECTION-2: Behavioral, Life style Factors and Diabetes Knowledge Questionnaire

S.No	Questions	Responses		
		Yes	**No**	**I don't Know**
1	Diabetes is caused by failure of the kidneys to keep sugar out of the urine			
2	Diabetes can be cured.			
3	Do have frequent medical checkup			
4	Do you drink alcohol?			
5	Have you ever been smoke tobacco product?			
6	Do you do any vigorous-intensity sports, fitness activities that cause large increases in breathing or heart rate like running or football, local dancing for at least 30 minutes continuously?			
7	Take diabetes medication as prescribed			
8	Follow specialist's dietary recommendations			
9	A fasting blood sugar level of 210 is too high			
10	check blood sugar levels frequently enough			
11	Diabetes can damage my kidneys			
12	Diabetes often causes poor circulation			
13	Duration of diabetes in years			
14	Comorbidities	1. Hypertension 2. Kidney disease 3. Other 4. No associated disease		
15	Concomitant drugs	1.Antihypertensive 2.lipid lowering 3.Weight reducing 4.Others		

SECTION-3: Anthropometric and Biochemical profile

Participant's identification code: _____ Date _____

Anthropometric measurement (filled by nurse)				
S.No	Parameters		Result	Laboratory personnel comment
1	Height		Cm	
2	Weight		Kg	
3	Waist circumstance		Cm	
4	WtHR			
5	BMI		Kg/m2	
6	BP		mmHg	
WSUTRH Laboratory Request Form for Participants (filled by Lab technologist)				
1	FBS		mg/dL	
2	**Lipid profile**	T.Cholestrol	mg/dL	
3		HDL	mg/dL	
4		LDL	mg/dL	
5		Triglyceride	mg/dL	
6	Creatinine		mg/dL	
7	HbA1c		%	

Reported by: Name of lab technologist_____ Date of report_____ Signature___

Annex 3 (B): Structured Questionnaire for Diabetic Study participants (Amharic Version)

የተሳታፊዉ ሚስጥራዊ ቁጥር፡- ---------- ቀን----------

ለክቡራን ተሳታፊዎች ከዚህ በታች የተዘረዘሩት ጥያቄዎች ከእናንተ የሚፈለጉ ናቸው፡፡ጥያቄው በአዲስ አበባ ዩኒቨርስቲ ጤና ሳይንስ ኮሌጅ በህክምና ላቦራቶሪ ድፓርትመንት ለድህረ ምረቃ ጥናት የሚያስፈልጉ ናቸው፡፡ የዚህ ጥናት አላማዉ በደረጃ ሁለት ስኳር ታማሚወች ላይ የደምስኳር፤ ኩላሊት ህመም ሁኔታ እና ኮለስትሮል መጠን ማጥናት ነው፡፡የእናንተ መልስ መስጠት ለዚህ ጥናት አላማ ዕውን መሆን አስፈላጊ ነው፡፡

ለትብብርዎ እናመሰግናለን!!!

ክፍል አንድ: የተሳታፊዎች አጠቃላይ መረጃ

ከዚህ በታች እርስዎን በትክክል የሚገልጽዎትን ክብ ያድርጉ

ተ.ቁ	ጥያቄ	መልስ
1	ዕድሜ	_____ዓመት
2	ፆታ	ሀ. ወንድ ለ. ሴት
3	የትዳር ሁኔታ	ሀ. ያገባ/ች ለ. ያላገባ/ች ሐ. የተፋታ/ች መ. የሞተበት(ባት)
4	ሐይማኖት	ሀ. ኦርቶዶክስ ለ. ፕሮተስታንት ሐ. ሙስሊም መ. ለሎች…….
5	የትምህርት ደረጃ	ሀ. የቀለምት/ትያልተማሩ ለ. አንደኛደረጃ ሐ. ሁለተኛደረጃ መ. ዲፕሎምና ከዛ በላይ
6	የመኖሪያ አድራሻ	1.ገጠር 2.ከተማ
7	የተሰማሩበት የስራ መስክ	1.የመንግስት ሰራተኛ 2.ነጋደ 3.ግብርና 4.የቀን ሰራተኛ 5.ሌላ……

ክፍል 2፡ስለ አጋላጭ ሁኔታወችና ስለሰኩዋር በሽታ ያለወት ግንዛቤ

ተ.ቁ	ጥያቄ	መልስ		
		አወ	አይ	አላውቅም
1	የስካር ህመም የሚከሰተዉ ኩላሊት በሰዉነታችን ዉስጥ ያለዉን የስካር መቆጣጠር ባለመቻሉ ነዉ			
2	ከስኩር ህመም መዳን ይቻላል			
3	የህክምና ክትትልወን በየጊዜዉ ያደርጋሉ			
4	አልኮል መጠጥ ይጠቀማሉ			
5	የትምባሆ ዉጤቶችን ይጠቀማሉ			
6	ስፖርታዊ እንቅስቃሴ በሳምንት ዉስጥ በየቀኑ ለ30 ደቅቃያህል ያለመቐረጥ ያደርጋሉ			
7	መድሀኒትወን በሀኪምወ ትዕዛዝ ነዉ ሚወስዱት			
8	በስፔሻሊሰት ሀኪምወ የተነገርወትን አመጋገብ ስርአት ይከተላሉ			
9	ምግብ ሳይበላ በፊትያለዉ የስካርመጠን ከ 210 ከበለጠ በጣምከፍተኛነዉሚባለዉ			
10	የስኩርወን መጠን በየጊዜዉ ይከታተላሉ			
11	የስካር ህመም ኩላሊትን ይጎዳል			
12	የስካር ህመም የደም ዝዉዉርን ያዛባል			
13	የስኩር ህመም ምን ያክል ጊዜ ቆየብወት			
14	ተጉዋዳኝ ህመም ካለብወት የትኛው	1.የደምግፊት 2.የኩላሊት 3.ሌላ 4.የለብኝም		
15	ለሌላ ህመም ሚወስዱትመድሀኒት ካለ	1.ለደም ግፊት 2.ቅባት መቀነሻ 3.ክብደት ለመቀነስ 4.ሌላ		

Annex 4: Laboratory SOPs

Mindray BS-200 chemistry analyzer

This instrument has been designed to perform spectroscopic measurement at predetermined wavelengths of analyte concentrations and enzyme activity using various reagents. We can perform any combination of tests up to 36-sample pipetting, incubations, photometric measurements and calculations. Programming and operating the analyzer is simple and made easy by windows software. The software, which is supplied with the analyzer, should be installed on a PC connected to the instrument.

Its sophisticated software allows us to program and permanently store in the memory of your PC almost unlimited number of tests and up to 40 test profiles, calibrators and controls. We can create routine sample request by assigning patients data and test and / or profiles to sample. Once the results have been obtained, you can request reports organized per patient or per test or examine the quality control data.

The analyzer can perform end-point or equilibrium method (one or two reagents, monochromatic or dichromatic), fixed time reaction (namely, first-order kinetic method or initial rate method) and kinetic mode method (namely, zero-order kinetic or continuous-monitoring method). The analyzer provides two calibration methods: linear calibration and non-linear calibration.

The linear calibration includes one-point linear calibration, two-point linear calibration and multi-point linear calibration. They are mainly used for tests determined by colorimeter.

The non-linear calibration includes Log it-Log 4P, Log it-Log 5P, Exponential 5P, Polynomial 5P, Parabola and Spline. They are mainly used for tests determined by turbidity.

Venous blood collection procedure

1. Introduce yourself and identify the patient

2. Explain the procedure to the patient

3. Wash hands and wear gloves

4. Prepare materials (syringes, needles, test tubes etc.)

5. Prepare the patient and apply tourniquet

6. Disinfect the draw site

7. Collect 5ml of blood with either Vacutainer tubes or syringe and needle

8. Exit the pain, apply pressure and check the patient.

9. Discard the needle in safety box

10. Label the specimen in each tube

11. Allow the specimen for 30minutes (to facilitate clotting) and centrifuge with medium speed for 5minutes

12. Separate serum from the blood by Pasteur pipette

13. Perform lab. Test according to the manufacturer's manual and store the remaining serum at -20 ^{o}C.

Annex-4.1. Principles of Laboratory analysis and procedures

Biochemical tests such as HbA1c is analyzed by Siemens dimension 200, fasting blood Glucose; Lipid profile, Creatinine and urea level are analyzed by using Mindray BS-200 fully automated clinical chemistry analyzer according to the manufacturer's instructions and procedures in WSUTRH laboratory. The instrument Mindray BS-200 chemistry analyzer is calibrated using Autocal and quality control samples both normal (Humatrol N) and pathological (Humatrol P) are run each day before running Patients samples.

1. Measurement of serum total cholesterol (TC)

Principle

Cholesterol is determined by a timed-endpoint method. Cholesterol esters are hydrolyzed to free cholesterol by cholesterol ester hydrolase (CE). The free cholesterol produced is oxidized by cholesterol oxidase (CO) to cholest-4-ene-3-one with the simultaneous production of hydrogen peroxide, which oxidatively couples with 4-aminophenazone and phenol in the presence of peroxidase to yield a chromogen. The absorbance is measured at 400 nm.

The reaction sequence is as follow:

Cholesterol ester + H_2O $\xrightarrow{CHE}$ Cholesterol + FFA

Cholesterol + O2 $\longrightarrow$ 4-Cholestinone + H_2O_2

2 H_2O_2 + 4-aminoantipyrine +phenol $\xrightarrow{POD}$ Quinonemine dye + $4H_2O$

Reagent

The reagents are standard and ready for use on automated analyzer. Enzymatic assay was adjusted at 400 nm in wavelength, 1 cm in optical path, 37 ^oC in temperature and measurement done against reagent blank. PIPES-200mmol/L(PH-7.0),Sodium cholate-1mmol/L, Cholesterol ester >250U/L, Cholesterol oxidase>250U/L,Phenol-4mmol/L->1KU/L,4-aminoantipyrine-0.33mmol/L,Phenol-4mmol/L,Non-ionic surface-2 g/l, Biocides.

Procedure: Samples, standard and reagent blank were pre-incubated for 5 minutes at 37 °C. Samples (10 µL) or standard (10 µL) and reagent blank (1000 µL) were pipetted into cuvette and mixed thoroughly by inversion. The cuvettes were inserted into the cell holder and stopwatch was started to count. The absorbance of sample, standard and the reagent blank was measured at 400 nm within 60 minutes. Finally the absorbance of the sample (ΔA sample) and the standard (ΔA standard) against the reagent blank was calculated.

$$TC(mg/dl) = \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} * C \text{ Standard}$$

2. Measurement of serum Triglyceride (TG)

Principle

Triglyceride (TG) level is estimated by a timed-endpoint method. TG measured using a series of coupled reactions in which triglycerides are hydrolyzed to produce glycerol and free fatty acids by the action of enzyme lipase. The glycerol formed phosphorylated to glycerol-3-phosphate by glycerol kinase. The glycerol-3-phophate is oxidized by glycerol phosphate oxidase producing dihydroxyacetone phosphate and hydrogen peroxide. Then, Peroxidase catalyzes the redox-coupled reactions of H_2O_2 with 4-aminoantipyrine (4-AAP), producing a bright purple color. The absorbance measured at 540 nm.

The reaction sequence is as follows:

Triglycerides + $3H_2O$ $\xrightarrow{\text{LPL}}$ Glycerol + fatty acid

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

Glycerol -3-phosphate +O_2 $\xrightarrow{\text{Glycerol phosphate oxidase}}$ DHAP +H2O2

H_2O_2 + 4-aminoantipyrine $\longrightarrow$ Quinonemine + H_2O

Reagents: The reagents are standard and ready for use on automated analyzer. Enzymatic assay and measurement was done at 540 nm wavelengths, 1cm optical path, and 37 ^oC temperature against reagent blank.

Reagent(R): phosphate buffer (PH 7.5)-100mmol/l,LPL-1500 U/L,GK-800 U/L,G-3-P oxidase -4000 U/L, Peroxidase -440 U/L, Phenol – 7.5 mmol/L, 4-aminoantipyrine – 0.7 mmol/L,ATP – 0.3mmol/L.

Procedure: Samples, standard and blank were pre-incubated for 5 minutes at 37 °C. Reagent blank (1000 µL) and samples (10 µL) or standard (10 µL) was put into cuvette and mixed gently by inversion. The cuvettes were inserted into the cell holder and stopwatch started to count. The absorbance of sample, standard and blank was measured at 540 nm. Finally, the absorbance of the sample (ΔA sample) and the standard (ΔA standard) against the reagent blank will be calculated.

$$TG(\text{mg/dl}) = \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} * \text{C Standard}$$

3. Measurements of serum high density lipoprotein cholesterol (HDL-C)

Principle

VLDL-c and LDL-c from serum are precipitated by phosphotungstate in the presence of magnesium ions. After removal by centrifugation, the clear supernatant containing HDL fraction and their cholesterol content was determined enzymatically.

The reactions are as follows:

Cholesterol ester + H_2O $\xrightarrow{\text{chol.estarse}}$ Cholesterol + FFA

Cholesterol + ½ O_2 + H_2O $\xrightarrow{\text{chol. Oxidase}}$ Cholestenone + H_2O_2

2 H_2O_2 + 4-Aminoantipyrine + DSBmT $\longrightarrow$ Quinonemine + $4H_2O$

Reagents

Reagents are standard and ready for use on automated analyzer. Enzymatic assay was done at 593 nm wavelength, 1 cm optical path, 37 °C temperature and measurement was done against reagent blank. PIPES-200mmol/L(PH-7.0),Sodium cholate-1mmol/L, Cholesterol ester

>250U/L, Cholesterol oxidase>250U/L,Phenol-4 mmol/L->1KU/L, 4-aminoantipyrine-0.33 mmol/L,Phenol-4 mmol/L, Non-ionic surface-2 g/l, Biocides.

Procedure

Reagent blank, samples and calibrator were pre-incubated for 5 minutes at 37 °C. Reagent blank (10 µL distilled water and 750 µL enzymes) and samples (10 µL samples and 750 µL enzymes) or calibrator (10 µL calibrator and 750 µL enzymes) was pipetted into cuvette and mixed gently by inversion. The cuvettes were inserted into the cell holder and stopwatch were started to count. The absorbance of sample, standard and the reagent blank were measured at 593 nm after 5 minutes. Finally the absorbance of the samples (ΔA sample) and the calibrator (ΔA standard) against the reagent blank was calculated.

$$\text{HDL(mg/dl)} = \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} * \text{C Standard}$$

4. Measurement of serum low density lipoprotein (LDL)

Most of the circulating cholesterol is found in three major lipoprotein fractions: very low density lipoproteins (VLDL), LDL and HDL.
[Total chol] = [VLDL-chol] + [LDL-chol] + [HDL-chol]

LDL-cholesterol is calculated from measured values of total cholesterol, triglycerides and HDLcholesterol according to the relationship: [LDL-chol] = [total chol] - [HDL-chol] - [TG]/5 where [TG]/5 is an estimate of VLDL-cholesterol and all values are expressed in mg/dL.

5.Measurement of fasting blood glucose

Principle: Fasting glucose is measured by GOD-PAP enzymatic method with deproteinization on a fully automated Mindray BS-200 clinical chemistry analyzer. Glucose present in the serum is oxidized by the enzyme glucose oxidase (GOD) to gluconic acid with the liberation of hydrogen peroxide, which is converted to water and oxygen by the enzyme peroxidase (POD). 4-aminoantipyrine [4-AA], an oxygen acceptor, takes up the oxygen and together with phenol

forms a red colored chromogen proportional to the concentration of glucose in the sample can be measured at 500nm(480 – 520 nm)

Procedure: 1. Separate the sample from whole blood

2. Write patient's demographic history and the type of test to be ordered on the work list

3. Put the reagent into the appropriate reagent disk

4. Put the sample tube into the appropriate sample disk

5. Order the machine according to the ordered test

6. **Hemoglobin A1c Measurement**

whole blood is treated with lysing reagent and is then mixed with the anti-HbA1c antibody in a buffered reagent. The HbA1c in the sample forms a soluble complex with the anti-HbA1c. A poly-hapten reagent containing multiple HbA1c epitopes is then added to the sample, forming an insoluble complex with the excess free anti-HbA1c antibody. This antibody poly-hapten complex is then measured turbidimetrically at 340 nm. Two ratio calculation options are provided to the customer in the package insert. The first option reports the results in % HbA1c while the second option reports the results in SI units of mmol/mol total hemoglobin.

7. **Creatinine Measurement**

   Creatinine under alkaline conditions reacts with picrate ions forming a reddish complex. The formation rate of the complex measured through the increase of absorbance in a prefixed interval of time is proportional to the concentration of Creatinine in the sample.

Creatinine +Alkaline picrate $\longrightarrow$ Orange colored complex

$\frac{\Delta A\ sample - \Delta A\ blank}{\Delta A\ standard - \Delta A\ blank} \times$ 2(Standard conc.) = mg/dL of (Creatinine in Sample)

Conversion factor : mg/dL × 88.4 = µmol/L

Declaration

I, the undersigned declare that this thesis complies with the regulations of the University and meets the accepted standards with respect to originality and quality. I also agree to accept responsibility for the scientific ethical and technical conduct of the research project and for provision of required progress reports.

M.Sc. candidate: **Eyouel Shimeles (BSc)**

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisors:

Mistire Wolde (MSc, PhD)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Mekdes Alem (MSc)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia