CARDIAC TROPONIN-I STATUS AND ASSOCIATED FACTORS IN TYPE-2 DIABETIC PATIENTS ON ANTIDIABETIC DRUGS TREATMENT AT JIMMA MEDICAL CENTER, JIMMA, SOUTHWEST ETHIOPIA

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A Thesis Submitted to Department of Biomedical Sciences, Faculty of Medical Sciences, Institute of Health, Jimma University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Medical Biochemistry

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Declaration

This is to certify that a thesis paper prepared by Alemayehu Babusha entitled *"Cardiac Troponin-I Status and Associated Factors in Type-2 Diabetic Patients on Anti-diabetic Drug Treatment at Jimma Medical Center, Jimma, southwest Ethiopia"* and Submitted in the Partial Fulfillment of the Requirements for the Degree of Master of Science in Medical Biochemistry complies with regulation of Jimma University and meets the accepted standards regarding originality and quality.

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ABSTRACT

Background: Vascular dysfunction caused by metabolic abnormalities in patients with type-2 diabetes mellitus is associated with accelerated atherosclerosis, myocardial infarction (MI), stroke, and increased risk of coronary heart disease. In spite of all these complications, there is no literature revealing the cardiac status and associated factors using cardiac troponin as a biomarker in study area and Ethiopia too. This study aimed to assess cardiac troponin-I and associated factors in type-2 diabetic patients on anti-diabetic drug treatment at Jimma Medical Center, Jimma, Southwest Ethiopia.

Methods: Facility based cross-sectional study was conducted among type-2 diabetic patients on follow-up at Jimma Medical Center chronic illness clinic from August 1 to September 30/2020. Systematic sampling technique was used to select 82 study participants, and face-to-face interview was carried out using semi-structured questionnaires during data collection. Five milliliters of venous blood was drawn from each participant after overnight fasting using an aseptic technique and centrifuged at 3000 rpm for 10 minutes. Multiple logistic regression analysis and one way ANOVA were used for statistical data analysis. P-value <0.05 was considered as statistically significant.

Result: The mean age of the subjects was 53.41± 13.85 years with a range of 23-85 years. The prevalence of elevated cardiac troponin-I was 25.6 % in the study population. Age greater than or equal to 60 years (AOR=13.735, 95% CI= 2.849-16.622, P= 0.013), SBP (AOR= 2.004, 95% CI=2.000-4.455, P=0.022), TC (AOR= 6.022, 95% CI= 1.225-12.961, P=0.039), LDL (AOR= 2.416, 95% CI= 1.744-3.346, P= 0.018) and TG (AOR= 2.468, 95% CI= 1.032-5.903, P= 0.048) were predictors of elevated cardiac troponin-I. Metformin drug treatment (AOR= 0.015, 95% CI= 0.001-0.435, P=0.015) was negatively associated with cardiac troponin-I. The mean value of serum cardiac troponin-I was lower in patients who were receiving metformin monotherapy as compared to patients on insulin monotherapy (11.654 ± 1.6795 vs 20.573 ± 1.8402, P<0.01), and metformin +glibenclamide (11.654 ± 1.6795 vs 21.094 ± 2.4062, P<0.01).

Conclusion: About one fourth of the study participants had elevated serum cardiac troponin-I level. Older age, systolic blood pressure and dyslipidemia were positively associated with elevated cardiac troponin-I. Metformin significantly reduced serum cTnI levels as compared to insulin monotherapy and metformin + glibenclamide. Regular screening for cardiac injury using cardiac troponin-I is recommended for type-2 diabetic patients.

Keywords: Cardiac troponin; Type-2 diabetes mellitus; Anti-diabetic drug

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ABBREVIATIONS AND ACRONYMS

ABP ____ Arterial Blood Pressure

ACS ____ Acute Coronary Syndrome

ADA ____ American Diabetic Association

AGEs ____ Advanced Glycation End products

AMPK ____ Adenosine Monophosphate Kinase

ASK1 ____ Apoptosis Signal-regulated Kinase-1

ATP _____ Adenosine Triphosphate

BMI _____ Body Mass Index

Ca^{+} _____ Calcium ion

CAD _____ Coronary Artery Disease

CHD _____ Coronary Heart Disease

CTn _____ Cardiac troponin

CTnI ____ Cardiac Troponin I

CTnT _____ Cardiac Troponin T

CKD _____ Chronic Kidney Disease

COVID-19 ____ Corona Virus Disease-2019

DNA _____ Deoxyribo Nucleic Acid

FDA _____ Food and Drug Administration

GDM _____ Gestational Diabetes Mellitus

GIP _____ Glucose- dependent Insulinotropic Polypeptide

GLP-1 _____ Glucagon- Like Peptide -1

GLUT-1 _____ Glucose Transporter type-1

GLUT-4 _____ Glucose Transporter type-4

HbA1c _____ Glycated hemoglobin A1C

HBP _____ Hexosamine Biosynthesis Pathway

HF _____ Heart Failure

HFrEF _____ Heart Failure with reduced Ejection Fraction

IDF _____ International Diabetic Federation

IGT _____ Impaired Glucose Tolerance

IGF1 _____ Insulin like Growth Factor-1

JAK-STAT ____ Janus kinase- signal transducer and activator of transcription

JMC _____ Jimma Medical Center

JNK _____ Jun N-terminal kinases

K^+ _____ Potassium ion

K^+ ATP _____ ATP sensitive potassium channel

LDL _____ Low Density Lipoprotein

LV _____ Left Ventricle

NADPH _____ Nicotinamide-adenine dinucleotide phosphate

NICE _____ National Institute Center Excellence

NIDDM _____ Non-Insulin Dependent Diabetes Mellitus

NLRP3 _____ Nucleotide binding domain Like Receptor Protein 3

OGT _____ Oral Glucose Tolerance

O-GlcNA _____ O-linked GlcNAc or O-linked β-N-acetylglucoseamine

PI3Kα _____ Phospho Inositide 3-Kinase Alpha

PKC _____ Protein Kinase C

PPAR- α _____ Peroxisome Proliferator-Activated Receptor alpha

P38-MAPK _____ P38 Mitogen Activated Protein Kinase

ROS _____ Reactive Oxygen Species

SERCA2a _____ Sarcoplasmic/endoplasmic reticulum Ca^{2+}ATPase 2a

STEPS _____STEPwise approach to surveillance

Sus _____ Sulfonylureas

VLDL _____ Very Low Density Lipoprotein

1. INTRODUCTION

1.1 Background

Diabetes mellitus is a chronic metabolic disease that is characterized by elevated blood glucose due to insulin resistance or insulin deficiency. This elevated blood sugar is responsible for triggering symptoms such as polyuria , polydipsia, and polyphagia (1).

Diabetes can be classified into four types, each having a different cause and management strategy. These include type-1 diabetes (due to autoimmune beta-cell destruction, leading to absolute insulin deficiency), type-2 diabetes (due to a progressive loss of beta-cell insulin secretion frequently on the background of insulin resistance), gestational diabetes mellitus, and other specific types of diabetes. However, the most common forms of diabetes are type-1 diabetes and type-2 diabetes which are heterogeneous diseases in which clinical presentation and disease progression may vary considerably(2).

According to the International Diabetes Federation(IDF) report, type- 2 diabetes became the leading type among all cases of diabetes mellitus, contributing to 90% to 95% of cases in developed countries and may exceed this percentage in developing countries due to urbanization and westernization (3).

Type-2 diabetes mellitus is associated with increased risk for cardiovascular disease, probably due to its nature of pathogenesis and coexisting cardiovascular risk factors like hypertension, obesity, and dyslipidemia(2). Vascular dysfunction caused by metabolic abnormalities in patients with type-2 diabetes is associated with accelerated atherosclerosis and increased risk of myocardial infarction (MI), stroke, and peripheral arterial disease. It is also a strong risk factor for coronary artery disease, and patients with diabetes have two to a four-fold greater risk of developing coronary artery disease(2,4).

Cardiac troponin is one of the cardiac biomarkers used to diagnose myocardial injury both in symptomatic and asymptomatic individuals(5). Troponin is a protein complex made up of three subunits namely troponin-T(TnT),troponin-I (TnI), and troponin-C(TnC) each having different structure and function and located on the thin filament of striated muscles. These three units of troponin complex along with tropomyosin is located on the actin filament and is essential for the calcium-mediated regulation of skeletal and cardiac muscle contraction (6,7).

Among the three troponins (TnT, TnC and TnI) TnT and TnI are being used as the biochemical markers for the diagnosis of myocardial injury. The amino acid sequence of cardiac troponin-T and troponin-I is different from that present in skeletal muscles. This is a base for cardiac troponin specificity in the diagnosis of myocardial injury(7). Normally, about 94-97% of these troponins are bound to myofibril and only 3% of the cTnI and 6% of cTnT is free in the cytoplasm, but during myocardial damage, the cytosolic troponin reaches the bloodstream quickly resulting in a rapid peak of serum troponin observed during the first few hours. Next, the release of structurally bound troponin results in a second peak lasting for several days. These detectable serum levels of cTn are an indicator of cardiac muscle damage(8,9).

Compared to other available cardiac markers, cardiac troponin-I has better sensitivity and tissue specificity and it detects myocardial injury. Following myocardial cell injury caused by myocardial ischemia, the myocardial cell membrane integrity is compromised and free cardiac troponin-I from myocardial cell cytoplasm is rapidly released into the blood circulation(10).

Drug treatments for diabetes mellitus are required when lifestyle modification alone fails to improve glycemic control and reduce the risk of cardiovascular complications(11). Metformin, insulin secretagogues, and acarbose are first- line oral glucose control therapies in type-2 diabetic patients. Whereas dipeptidyl peptidase-4 inhibitor, a thiazolidinedione, and glucagon-like peptide-1 receptor agonists are second-line drugs introduced when blood glucose levels can’t be controlled by first-line drugs or when patients are unable to tolerate those drugs. The final stage is insulin which is used alone or in combination with other oral hypoglycemic drugs in case blood glucose level control can’t be achieved by the drugs mentioned above(11,12).

1.2 Statement of the Problem

Diabetes mellitus is currently considered as a public health problem, and its incidence and prevalence are increasing worldwide(13). The report of the International Diabetes Federation (IDF), in 2019 indicated that diabetes mellitus caused 4.2 million deaths; and 463 million adults aged between 20 and 79 years old were living with diabetes. From this projection, it is estimated that the number will likely rise to 700 million by 2045(14). The major type of diabetes epidemic is type-2 which accounts for about 90% to 95% of all diagnosed cases of diabetes mellitus(15). The number of people with type-2 diabetes is increasing due to population growth, aging, urbanization, and increasing prevalence of obesity and physical inactivity(15).

There is a variation of incidence and prevalence of type-2 diabetes mellitus according to the geographical region, with more than 80% of patients living in low to middle-income countries(14); this thought to be due to the adoption of western life styles and urbanization (13). In Sub-Saharan Africa, the average number of adults with type-2 diabetic cases was 15.5 million in 2017, with a regional prevalence of approximately 6%, and this number is estimated to increase by 162.5% by 2045(16).

Like other Sub-Saharan African countries, Ethiopia is suffering from a high burden of diabetes, with increased prevalence, complications, and mortality and life-threatening disabilities. According to a report of World health organization (WHO) in 2000, the number of diabetes cases in Ethiopia was 800,000. Meanwhile, this number is estimated to rise to 1.8 million by the year 2030 (16,17).

World Health Organization's, report revealed that a trend in the prevalence of diabetes in Ethiopia has been increasing over the past years. According to the 2017 International Diabetes Federation(IDF), the prevalence of type-2 diabetes mellitus in Ethiopian adults was 5.2%(15).

Diabetes mellitus is a common risk factor for cardiovascular disease. People with type-2 diabetes mellitus have higher cardiovascular morbidity and mortality. Diabetic vascular disease is responsible for a two to a four-fold rise in the occurrence of coronary artery disease (CAD) and stroke, and a two to eight-fold increase in the risk of heart failure(18). Heart disease is common in people with diabetes. In 2012, American National Heart Association

(ANHA) reported that heart disease and stroke account for 65% of diabetic-associated mortality worldwide(19). Other studies found that the likelihood of death from heart disease or stroke increases by more than twice in diabetic patients(19,20).

Diabetes mellitus is a major contributor to various heart diseases in Ethiopia, including ischemic heart disease, left ventricular hypertrophy, and reduced systolic and diastolic function. It is the strongest predictor of an incident of heart failure and those with both diabetes and hypertension are at the highest risk(21).

Medications used in the treatment of type-2 diabetes mellitus may have various cardiovascular effects (harmful, beneficial, or neutral). However, for the majority of the anti-diabetic drugs, their cardiovascular effects are not clear (22). Even though metformin is commonly used oral hypoglycemic agent and reduces cardiovascular risk factors such as body weight, fat and blood pressure, its effect on coronary heart disease remains controversial(23,24).

There are still contradictions in the research on sulfonylurea drugs and their cardiovascular safety. However, compared with metformin, the use of sulfonylurea significantly increases the risk of heart failure(25). Existing data for insulin in both clinical trials and observational studies are inconsistent concerning cardiovascular outcome(26).

Although the prevalence of type-2 diabetes mellitus is increased and accompanied by cardiovascular complications, there are no data showing cardiac status and associated factors using cardiac troponin-I as a biomarker in Ethiopia up to the researcher's knowledge. In addition, it has not been determined whether anti-diabetic drugs have different cardiovascular effects in type-2 diabetic patients in the Ethiopian population. Therefore, the purpose of this study was to assess the cardiac status using cardiac troponin-I, determine associated factors and compare the effects of different anti-diabetic drugs on cardiac troponin-I in the study area.

1.3 Significance of the Study

Patients with type-2 diabetes mellitus have a considerably higher risk of cardiovascular morbidity, mortality, and they are disproportionately suffering from its complications. Cardiac diseases like acute coronary syndrome (ACS), cardiomyopathy, and heart failure are

fatal cardiovascular complications attributed to type-2 diabetes mellitus mainly. Though the cardiovascular effect of type-2 diabetes was elucidated clearly, the cardiac status and associated factors were not determined using cardiac troponin-I in type-2 diabetic patients in Ethiopia to the level of the researcher's knowledge. The result of this study will provide information on the prevalence of elevated cardiac troponin-I and associated factors that enable health professionals to create awareness among patients in the study area. Conducting this study is also crucial for improving the early diagnosis and management of heart disease induced by type-2 diabetes mellitus. Comparing the effects of different anti-diabetic drugs on cardiac troponin-I may enable health professionals to choose safe anti-diabetic drugs for patients with type-2 diabetes at an early stage of treatment to prevent further cardiovascular complications.

On the other hand, the result of the study may help health planners and caregivers to conduct evidence-based interventions and guide future decision makers. Moreover, the finding of this study may serve as a baseline for further research. Hence, this study is aimed to assess cardiac troponin-I, identify associated factors and compare the effect of different anti-diabetic drugs on cardiac troponin-I in type-2 diabetic patients on anti-diabetic drug treatment attending their follow-up at the chronic illness clinic of Jimma Medical Center.

2. LITERATURE REVIEW

2.1 Diabetes Mellitus

Diabetes mellitus is a chronic metabolic syndrome characterized by abnormally high blood glucose resulting from defects in the secretion and action of insulin and impaired function in the metabolism of carbohydrates, lipids, and proteins which results in long-term health complications. Type-2 diabetes is a combination of low amounts of insulin production from pancreatic β-cells and peripheral insulin resistance(27).

Insulin resistance leads to the impairment of glucose transport into the muscle cells, subsequently leading to elevated hepatic glucose production. Further, it elevates the plasma level of free fatty acids and fat breakdown also. For type-2 diabetes to develop there must be insulin resistance and pancreatic β-cell dysfunction simultaneously(28).

2.1.1 Pathophysiology of Type2 Diabetes Mellitus

The main attribute of T2DM is insulin insensitivity, declining insulin production, and eventual pancreatic beta-cell failure(29). This results in a decrease in glucose transport into the liver, muscle cells, and fat cells. This leads to an increase in the breakdown of fat with hyperglycemia. The presence of impaired alpha-cell function has recently been recognized in the Pathophysiology of type-2 diabetes mellitus(30). Due to this dysfunction, glucagon and hepatic glucose levels that rise during fasting are not suppressed with a meal. Given inadequate levels of insulin and increased insulin resistance, hyperglycemia results(31).

A large proportion of individuals suffering from type-2 diabetes are obese, with central visceral adiposity. Therefore, the adipose tissue plays a crucial role in the pathogenesis of T2 DM(30,31). Under normal circumstances, plasma glucose levels are maintained within a narrow range, despite wide fluctuations in supply and demand, through a tightly regulated and dynamic interaction between tissue sensitivity to insulin (especially within the liver) and insulin secretion. In type-2 diabetes, these mechanisms break down, with the consequence that the two major pathological defects in type-2 diabetes are impaired insulin secretion through a dysfunction of the pancreatic β-cell, and impaired insulin action through insulin resistance (3).

2.1.2 Risk Factors for Type-2 Diabetes Mellitus

Several risk factors have been associated with the development of type 2 diabetes. Genetic factors in some ethnic groups, family history of diabetes, and increasing population age are examples of non-modifiable risk factors. Furthermore, lifestyle factors associated with an unhealthy diet, physical inactivity, smoking, overweight, dyslipidemia, high blood pressure, and impaired glucose tolerance (IGT) are the most common risk factors for escalating diabetes epidemiology(32).

2.1.3 Diabetes and Cardiovascular Disease

Patients with type-2 diabetes are still at increased cardiovascular risk and cardiovascular disease remains the leading cause of death in T2DM. A scientific statement from the American Heart Association and the American Diabetes Association explains that for every 18 mg/dl (1mmol/L) increase in fasting plasma glucose, the risk of future cardiovascular events or death increases by17%. One percent (1%) rises (11mmol/mol) in HbA1c results in an 18% increase in the risk of cardiovascular events. Furthermore, an analysis of eleven large, integrated health care organizations in the United States found an increased Cardiovascular risk if HbA1c was ≥9%(75mmol/mol)(33).

The European Prospective Investigation of Cancer and Nutrition (EPIC)-Norfolk study found that an increase of 1% in HbA1c is associated with a 28% increase in the risk of all-cause death in men, independent of age, blood pressure, serum cholesterol, body mass index, and cigarette smoking(34).

Diabetic patients often exhibit silent cardiac dysfunction which is detectable only in the latter stage of the disease. Even ≈50% of individuals with well-controlled diabetes mellitus, asymptomatic and normotensive patients are considered to exhibit some degree of cardiac dysfunction(35).

Subjects with type-2 diabetes have over twice the risk of incident of heart failure than people without diabetes and heart failure is the most common initial presentation of cardiovascular disease due to all the major risk factors for heart failure also cluster in patients with type 2 diabetes, including obesity, hypertension, advanced age, sleep apnoea, dyslipidemia, anemia, chronic kidney disease (CKD), and coronary heart disease (CHD)(36,37).

2.1.4 Mechanisms Contributing to Diabetic Heart Disease

2.1.4.1 Oxidative Stress

NADPH oxidase, mitochondrial respiration, and uncoupled NO synthase are the major sources of reactive oxygen species (ROS) in diabetic patients. Besides increased ROS, endogenous anti-oxidants decrease(38,39).

Increased ROS is responsible for oxidative damage to (protein, lipid, and DNA), trigger inflammasome activation, and activates different pathological signaling like such as protein kinase C (PKC), apoptosis signal-regulating kinase-1(Ask1), p38 mitogen-activated protein kinase(p38-MAPK), JNK, and JAK-STAT pathways all of which cause diabetes mellitus-induced cardiac complications as well as induce ROS generation(40–42).

2.1.4.2 Inflammation

Systemic inflammation is present in Type-2 diabetic patients accompanied by increased circulating cytokines, chemokines, and immune cells. Besides, diabetes mellitus induces tissue infiltration of macrophages as well as their polarization toward M1-like proinflammatory phenotype in affected patients, with increased leukocyte inflammatory cytokine signaling(43,44).

The infiltration of the myocardium with immune cells contributes to myocardial injury, myocardial ischemia LV dysfunction, and heart failure with reduced ejection fraction (HFrEF)(45,46).The Proinflammatory state is mainly induced by ROS-triggered activation of nucleotide binding domain-like receptor protein 3 (NLRP3) inflammasome pathways(47) which triggers a cascade of events, recruiting pro–caspase-1 to facilitate caspase-1 activation, and cleavage of IL-1β and IL-18 precursors to generate their active products(48,49). This alters macrophage polarization toward M1-like phenotype and eventually results in diabetes mellitus–induced LV fibrosis and dysfunction (48,50).

2.1.4.3 Changes at the Level of Insulin Sensitivity and Signaling

Insulin resistance is associated with reduced glucose uptake to cardiomyocytes and a decrease in GLUT4 expression. In response to insulin phosphoinositide 3-kinase (PI3Kα), AKt mediates myocardial contractility, and vascular tone(51). In the diabetic heart, the effectiveness of endogenous physiological mechanisms such as insulin sensitivity and

downstream signaling, through both insulin and IGF1 (insulin-like growth factor-1) receptors (via activation of PI3Kα and its mediator AKt), has also been implicated (52).

2.1.4.4 Altered Cardiac Metabolic Pathways

In type-2 diabetic patients, there is reduced glucose oxidation, increased fatty acid oxidation, and reduced glycolysis. In addition, ROS-induced mitochondrial uncoupling in the diabetic myocardium markedly reduces cardiac efficiency(53,54). Glucotoxicity induced by advanced glycation end products (AGEs) and the hexosamine biosynthesis pathway (HBP) also cause myocardial dysfunctions (48,55).

There is also increased circulating levels of free fatty acid and other lipids due to impaired insulin action in adipose tissue and liver which is accompanied by altered substrate utilization for energy utilization relying on fatty acid at the expense of glucose utilization (56,57). An increase in myocardial lipid content leads to lipotoxicity and substrate preference abnormality at the level of mitochondria induces mitochondrial impairments(58). Mitochondrial ROS generation exceeds the endogenous scavenging capacity leading to cardiac oxidative stress and inflammation which may cause myocardial damage (58,59).

2.1.4.5 Impaired Calcium Handling

Putative mechanisms for impaired calcium handling include reduced gene and protein expression, or reduced activity, of SERCA2a, posttranslational modifications of this Ca2+ handling protein as a result of AGEs or O-GlcNAcylation and oxidative modifications (55,60,61).

Impaired Ca2+ influx and efflux, altered Ca2+levels in intracellular organelles (including both the sarcoplasmic reticulum and mitochondria), altered expression, and activity of other Ca2+-handling proteins, as well as myofibrillar responsiveness to Ca2+, are also defective in the diabetic heart(51). This spectrum of diabetes mellitus– induced defects in Ca2+ handling and responsiveness to Ca2+ underlie delays in cardiomyocytes lengthening in the diabetic heart and thus have been attributed to account for a significant component of diabetes-induced LV diastolic dysfunction(51,55,62).

2.1.4.6 Dyslipidemia

In type-2 diabetes, there is increased very-low-density lipoprotein (VLDL) cholesterol, triglycerides, and small and dense LDL cholesterol levels but decreased high-density lipoprotein (HDL) cholesterol levels. These elevated lipoproteins are modified by oxidation as well as glycation and lead to atherosclerosis(63).

Oxidized LDL also attract leukocytes to the intima of the vessel, improving the ability of the leukocytes to ingest lipids and differentiate into foam cells, and stimulating the proliferation of leukocytes, endothelial cells, and smooth muscle cells(64), all of which are steps in the formation of atherosclerotic plaque. In patients with diabetes, LDL particles can also Hypertriglyceridemia can lead to increased production of the small, dense form of LDL and to decreased HDL transport of cholesterol back to the liver(65).

Insulin resistance promotes dyslipidemia accompanied by increased oxidation, glycosylation, and triglyceride enrichment of lipoproteins. Moreover, endothelial dysfunction is present, and all of these factors contribute to the increase in atherogenicity, and macrovascular disease leading to heart disease(66,67).

2.1.4.7 Neurohumoral Mechanisms

Activated renin-angiotensin-aldosterone system in diabetes mellitus results in diabetes mellitus induced cardiac remodeling. Diabetes mellitus is also associated with autonomic dysfunction which leads to cardiac abnormality and increases in the level of immune cells, cytokines, and damage-associated molecular pattern molecules all of which exacerbate inflammation(68,69).

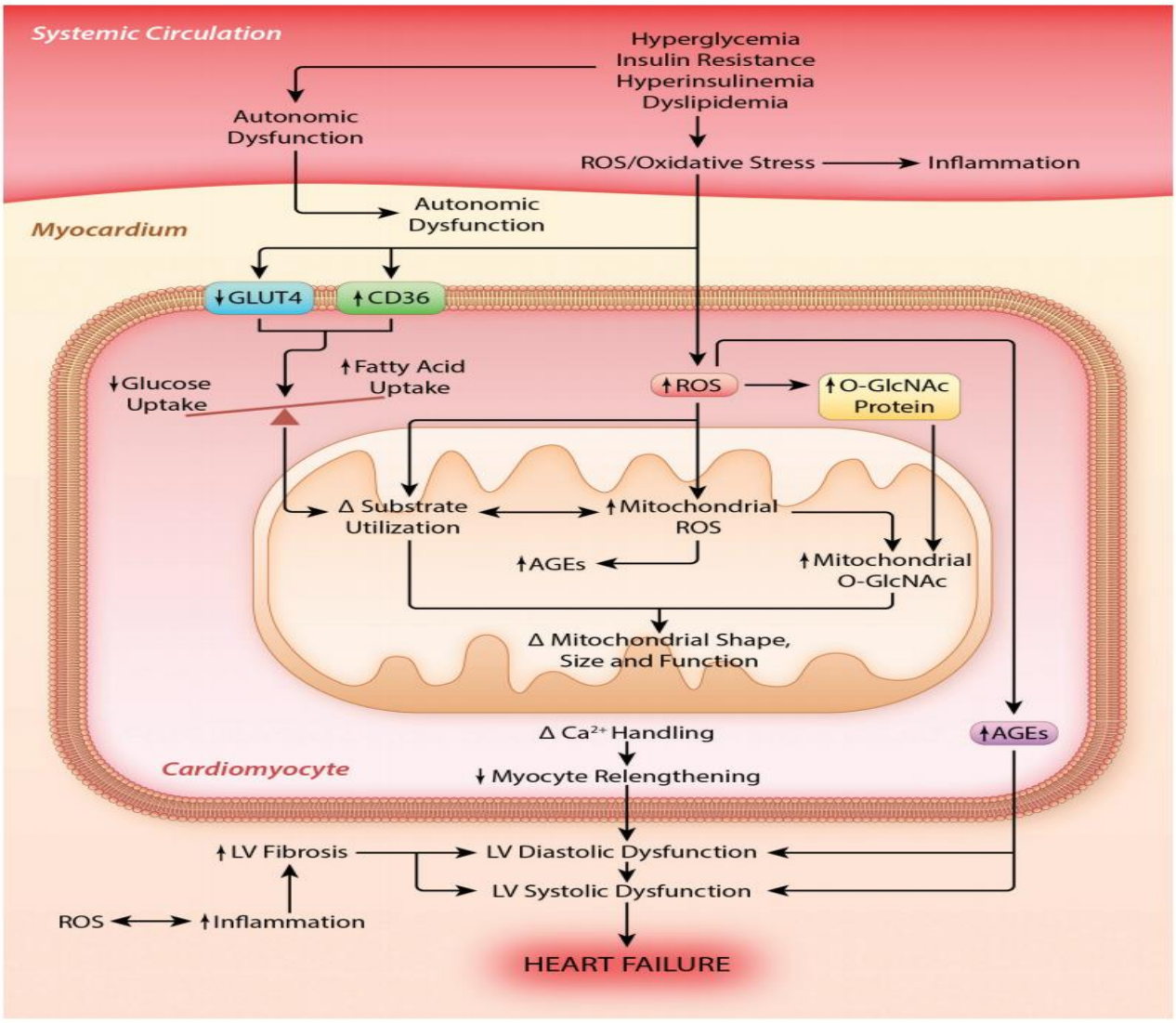

Figure 1: The contributing mechanisms to diabetes mellitus–induced cardiomyopathy(70)

2.2 Medical management of Type-2 Diabetes Mellitus (Anti-diabetic Drugs)

2.2.1. Sulfonylureas (glibenclamide, gliclazide, glipizide, glimepiride)

The mechanism of action of sulfonylureas involves the stimulation of the pancreatic islet beta-cells to secrete insulin. These drugs bind the adenosine triphosphate (ATP) sensitive potassium channels (K+ATP) on the cell membrane of pancreatic beta cells, which depolarizes the cell by preventing K+ from exiting and thus results in the opening of voltage-gated Ca2+ channels. The increase in intracellular Ca2+ leads to increased fusion of insulin granules with the cell membrane, and therefore, augmented secretion of mature insulin. This effect leads to the amplified responsiveness of β-cells to both glucose and non-glucose

secretagogues, resulting in more insulin being released and lower blood glucose concentrations. Due to their mechanisms of action, studies have warned its use in patients with acute myocardial infarction(71,72).

Sulfonylureas can be classified as first-or second-generation agents based on their potencies. First-generation drugs include acetohexamide, chlorpropamide, tolbutamide, and tolazamide. Currently using drugs from this class is not currently recommended due to their potential to elicit adverse effects. However, the second-generation, such as glipizide, gliclazide, and glibenclamide, known as glyburide and glimepiride, is the most commonly used internationally (73).

2.2.2 Biguanides (Metformin, Metformin-extended release)

The molecular mechanisms of action have not yet been established. However, it is thought that insulin sensitivity is improved and mediated via modification of post-receptor signaling in the insulin pathway. A protein, adenosine 5'-monophosphate protein kinase, has been identified as a possible target of metformin(74,75).

This class of drug increase hepatic insulin sensitivity, thereby reducing gluconeogenesis as well as glycogenolysis, which contribute to the post-prandial plasma glucose-lowering effects. Skeletal muscle and adipocytes increase the insulin-sensitive GLUT-4 and GLUT-1 transporters to the cell membranes, thereby increasing glucose uptake. Glucose metabolism in the splanchnic bed also increases. Further metabolic effects include suppression of fatty acid oxidation as well as triglyceride lowering(71,76).

2.2.3 Insulin

Insulin initiates its action by binding to a glycoprotein receptor on the surface of the cell membrane. This receptor consists of α-subunit, which binds the hormone, and a β-subunit, which is an insulin-stimulated, tyrosine-specific protein kinase. Activation of this kinase is believed to transmit sign that eventually leads to insulin's action on glucose, lipid, and protein metabolism(77,78).

Insulin is eventually required for several people with type- 2 diabetes and early initiation are often appropriate. Beta-cell function declines linearly and after ten years 50% of people with type 2 diabetes will require insulin. Insulin has a greater blood-glucose-lowering ability than

any other hypoglycemic medicine, and early initiation may reduce beta-cell damage and is thought to slow disease progression. Early initiation of insulin should be strongly considered for people with type-2 diabetes who have significant hyperglycemia, particularly if there are signs such as ketonuria and weight loss(20,79).

Insulin as added to metformin -based regimens are shown to enhance glycemic control, limit changes in weight , reduce hypoglycemia incidence, and limit insulin requirements (sparing effect), allowing a 15–25% reduction in total insulin dosage(80).

2.3 Troponin as a Cardiac Biomarker

Troponins are cardiac regulatory proteins that are found in the cytoplasm of cardiac myocytes. When calcium binds to the protein complex, the structure of troponin changes, and this causes an interaction between the actin and myosin filaments. This interaction leads to cardiac muscle contraction. The troponin complex is made up of three subunits: cTnC, cTnI, and cTnT(81).

CTnI and cTnT are the subunits that are identified in laboratory testing looking for cardiac muscle injury; cTnI is identified to be exclusive to cardiac muscle. Small amounts of cTnT have been identified in skeletal muscle but are found in much higher concentrations in cardiac muscle. In clinical studies, there have been no statistically significant differences found when using cTnI versus cTnT in troponin assays(82).

Myocardial ischemia is the first step in developing a MI and occurs as a result of oxygen supply-demand mismatch to or reduced coronary flow (83). The Pathophysiology underlying acute coronary syndrome (ACS) is atherosclerotic plaque rupture or erosion. This leads to progressive myocardial ischemia which, if sustained, leads to infarction via three possible mechanisms: (i) intra-luminal platelet aggregation resulting in partial or complete vascular occlusion;(ii) release of platelet micro aggregates which results in the micro-embolism of small vessels to cause localized ischemia and infarction; and (iii) progression of white thrombus formation to clotting cascade activation which results in partial or total occlusion of the epicardial artery(81).

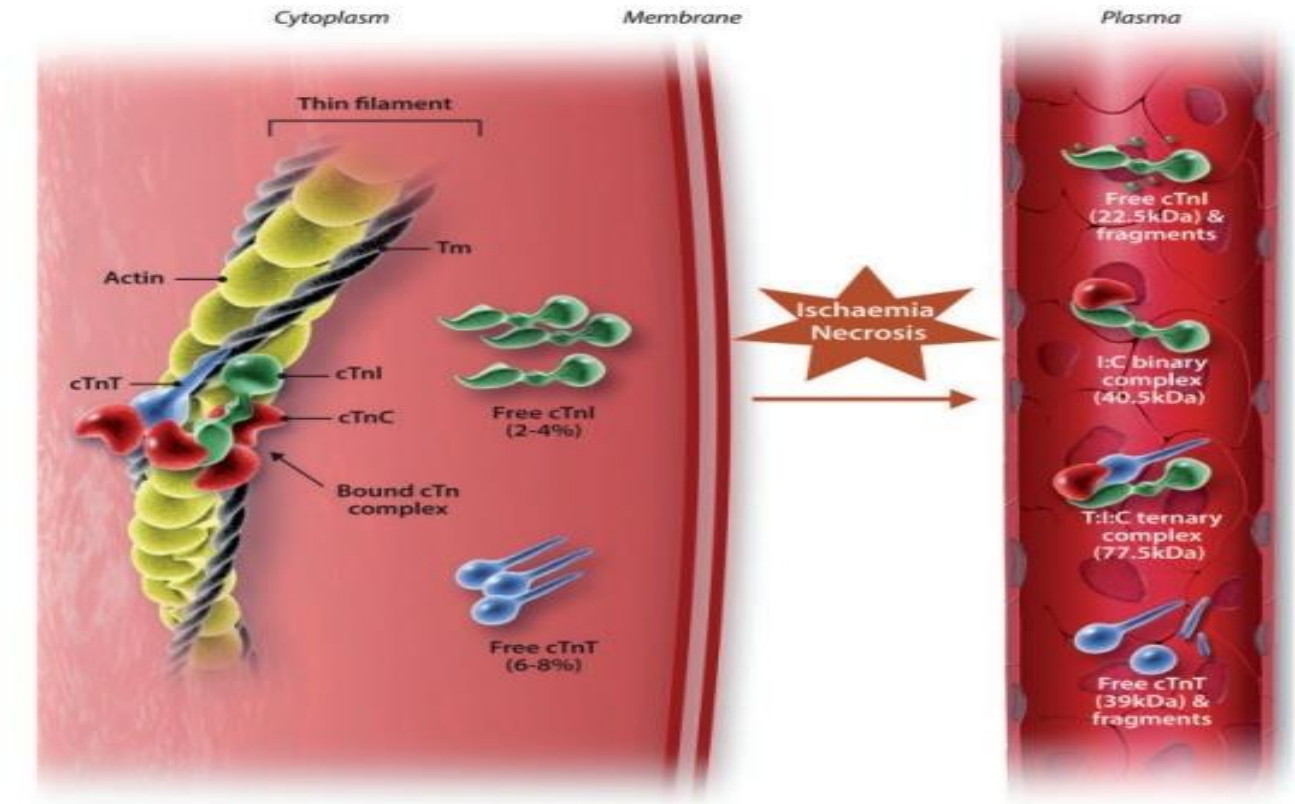

Figure2: Structure of the cardiac troponin-tropomyosin complex and the forms of troponin released following myocardial necrosis(81).

Both the European Society of Cardiology (2007) and the third universal definition of myocardial infarction(2012) recommended cardiac troponin (cTn) to support the diagnosis of AMI due to its good sensitivity and specificity(10).

2.4.1 Biochemical Mechanisms of Troponin Cleavage

Troponin is released from the myofibril due to proteolytic degradation in the myocardium, both as intact proteins and as degradation products(84). The three myocardial enzyme that degrade cardiac troponin are Calpain 1, Caspase, and a matrix metalloproteinase-2 (81). These enzymes are also present in blood and the complexes of cTnI (T: I:C and I: C complex) are susceptible to degradation in the circulation(84).

The N-and C-terminal regions of cTnI are the most susceptible to proteolysis. The central region of cTnI (residues 30-110) is the Ca^{+2}-dependent TnC binding domain and is the most stable. For this reason, this is the region currently targeted by most cTnI assays(85).

2.4.2 Anti-diabetic drugs and Cardiac Troponin

A Study reported by Harvard medical school on randomized trial of the effects of insulin and metformin on myocardial injury after 12 weeks follow up in type-2 diabetic patients have shown no significant change in high sensitive cardiac troponin(86) meanwhile study in the USA using high sensitive cardiac troponin-T(hscTnT) as a biomarker for subclinical myocardial injury after 6 months follow up revealed the association of insulin with higher high sensitive cardiac troponin-T(87). Another study conducted in Argentina on patients with the acute coronary syndrome of non–ST-segment elevation with type-2 diabetes revealed that cTnI levels were lower in both metformin treated group as well as glibenclamide treated group compared to control(88).

A systematic review and meta-analysis reported that metformin limits infarct-size and improves cardiac function in animal models of myocardial infarction(89). Additionally another study found that chronic metformin use was associated with lower peak values of cardiac troponin compared to non-metformin using patients(90).

In contrary to the above findings the American Heart Association reported no statistically significant association between metformin and myocardial infarct size using Troponin as a biomarker in patients with diabetes and acute ST-segment elevation myocardial infarction(91). A Study concerning sulfonylureas found that Sulfonylurea users had higher cTI compared to non-sulfonylurea users in patients with Type-2 diabetes mellitus and segment elevated myocardial infarction(STEMI)(92).

2.4.3 Factors Associated with high Cardiac Troponin

In a retrospective study conducted in Nepal Patients with chest pain and positive troponin test (with a confirmed cardiac event) was found to have significantly elevated levels of total cholesterol, triacylglycerol levels, low density-lipoprotein level, and significantly reduced high-density lipoproteins cholesterol levels when compared to the patients who experienced only chest pain (negative troponin) and healthy controls(93).

Additionally, a study in India reported the occurrence of myocardial infarction was associated with elevated troponin T levels; troponin T was positively correlated with total cholesterol, triglycerides, low density lipoprotein, and Total cholesterol to high density lipoprotein cholesterol ratio and negatively correlated with HDL. Total cholesterol to high

density lipoprotein cholesterol ratio was not found to be a useful predictor of the likelihood of MI(94).

Another study described that Higher hs-TnI concentration was associated with older age, male sex, and increased atherosclerosis burden(95). Male gender, HbA1c, and smoking status were independently associated with hs-cTnI concentration according to a study of a European multinational cohort in the presumably healthy population(96).

2.5 Conceptual Framework

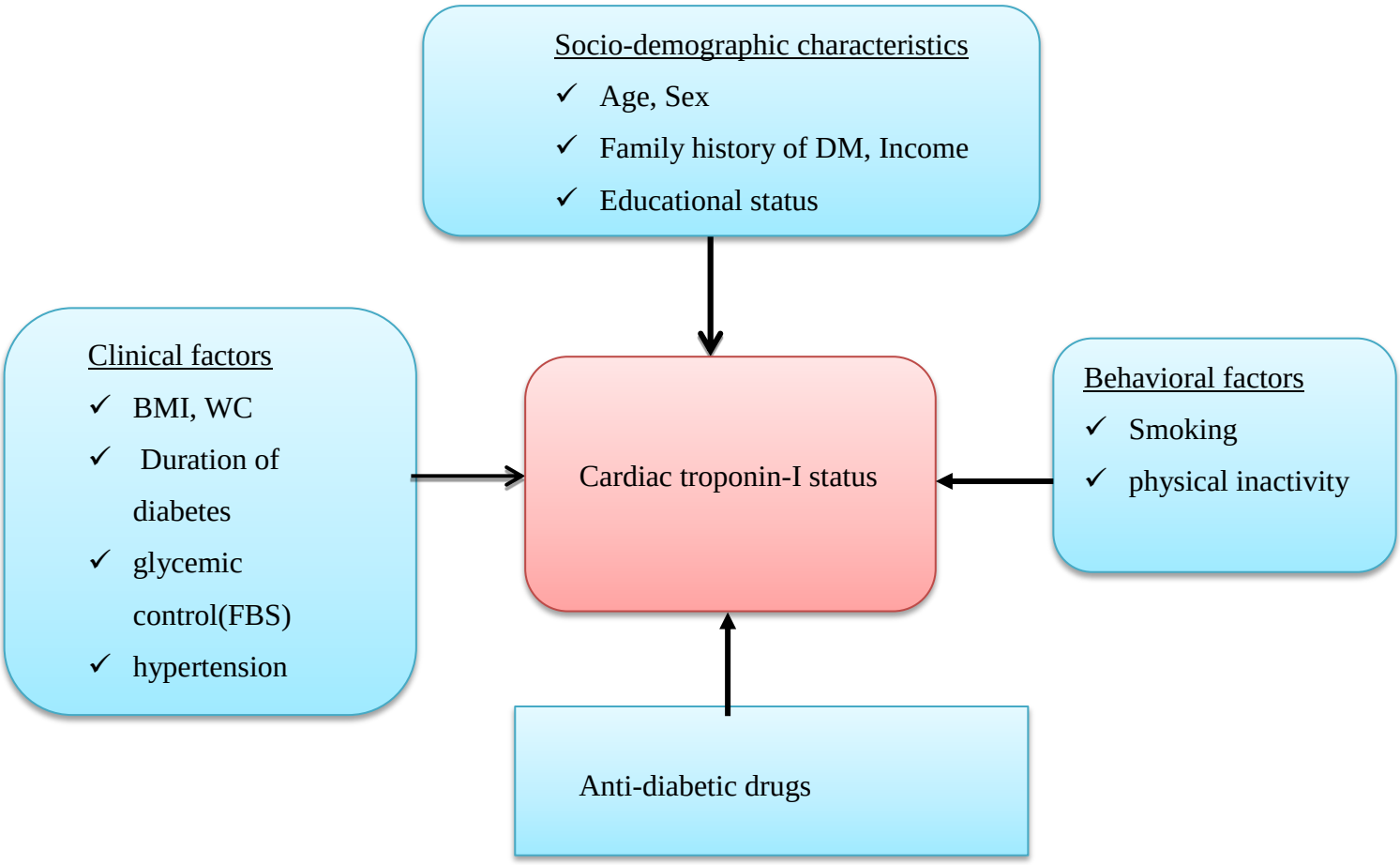

Figure 3 : Conceptual framework (29,32,36,37,93,95,96).

3. OBJECTIVES

3.1 General Objective

- To Assess the serum level of Cardiac Troponin-I and Associated Factors in Type-2 Diabetic Patients on Anti-diabetic Drug Treatment at Jimma Medical Center, Jimma, Southwest Ethiopia, 2020

3.2 Specific Objectives

- To determine the mean ± SD of serum level of cardiac troponin-I and the prevalence of elevated cardiac troponin-I levels in type-2 diabetic patients on different anti-diabetic drug treatment
- To assess the serum level of lipid profiles (LDH, HDL,TC and TG) among type-2 diabetic patients on anti-diabetic drug treatment
- To determine glycemic control through evaluating fasting blood glucose level in type-2 diabetic patients on anti-diabetic drug treatment
- To determine anthropometric indices and arterial blood pressure in type-2 diabetic patients on anti-diabetic drug treatment
- To evaluate factors associated with elevated serum cardiac troponin-I levels in patients with type-2 DM on different anti-diabetic medications in Jimma medical center

Hypothesis

HO: There is (are) no associated factor (s) on the serum level of cardiac troponin-I among T2DM patients on different antidiabetic medications at JMC, Jimma.

HA: There are associated factors on the serum level of T2DM patients on different antidiabetic medications at JMC, Jimma.

4. METHODS AND MATERIALS

4.1 Study Area and Period

The study was conducted at Jimma Medical Center (JMC) from August 1- September30 /2020. JMC is located in Jimma town at a distance of 352 km to the Southwest of Addis Ababa. It is the only teaching and referral hospital in the South-Western part of the country with a bed capacity of 600.

4.2 Study Design

A facility based Cross- sectional study was conducted

4.3 Population

4.3.1 Source Population

The source of population for this study was all type- 2 diabetic patients who were on anti-diabetic drug treatment and attending their follow-up at the chronic illness clinic of JMC

4.3.2 Study Population

The study population was all sampled type 2 diabetic patients who were attending follow-up at the chronic illness clinic and fulfilled the inclusion criteria

4.3.3 Inclusion and Exclusion Criteria

4.3.3.1 Inclusion Criteria

Type- 2 diabetic patients 18 and above years

4.3.3.2 Exclusion criteria

- Patients who have chronic kidney disease and patients who are critically sick and unable to communicate.
- Patients who were taking lipid lowering drugs
- Patients who were taking chemotherapy
- Pregnant women

4.4 Sample Size Determination and Sampling Technique

4.4.1 Sample Size Determination

The sample size was calculated by using single population proportion formula as follows

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

n= minimum sample size required

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

Using p=26% prevalence of elevated troponin in USA(97)

$n= (1.96)^2 x\ 0.26x0.74/0.05=296$

Using population correction formula nf= 296/1+296/2700=296/1.11=267

But due to budget constraint we used p=0.052%

P= National prevalence of type2 diabetes mellitus =0.052(98)

d=margin of error = 0.05

$n= (1.96)^2 x\ 0.052(0.948)/0.05^2= (3.8416)\ x0.049296/0.0025=75.75=76$

Taking population correction formula as

$n_f = \frac{n}{1+n/N}$ =76/1+76/2700=73.9=74 where 2700 is total T2DM patients on follow up.

Adding 10% non-response rate=74+7.4=82. Hence final sample size = 82

4.4.2 Sampling Technique

A systematic sampling technique was used. The k-value was calculated by dividing the total population by the sample size, K=2700/82=32.926=33. Data were collected from every 33 individuals starting from the first arrived patients on the beginning of data collection.

4.5 Study Variables

4.5.1 Dependent Variable

Serum cardiac troponin-I level

4.5.2 Independent Variables

- Socio-demographic characteristics (Age, Sex, income and educational status)
- Duration of diabetes mellitus
- Antidiabetic drugs
- Behavioral factors: Smoking, alcohol, and physical activity
- Lipid profiles(LDL, HDL, TC, and TG)
- Fasting blood sugar
- Arterial blood pressure, body mass index, and waist circumference

4.6 Data Collection Tools and Techniques

The instruments used for data collection were adapted mainly from the WHO‘s stepwise (STEPs) approach for non-communicable disease surveillance. STEPs is the WHO-recommended surveillance tool for chronic disease risk factors and chronic disease-specific morbidity and mortality which is intended to serve as an entry point for low and middle-income countries into surveillance of chronic diseases and their risk factors(99). This approach is characterized by the use of questionnaires to gain information on risk factors, simple physical measurements (anthropometric and blood pressure measurements), and biochemical measurements.

4.6.1 Questionnaires

Pre-informed written consent was obtained before running any data collection then, data collectors used semi-structured questionnaires for data collection in a face to face interview as well as document review. The questionnaires have four components: Questions on socio-demographic characteristics, behavioral risk factors, clinical risk factors, and biochemical measurements

4.6.2 Anthropometric Measurements

The height scale and the digital weighing machine were used to measure height and weight respectively. Subjects were weighed barefoot in very light clothing and body mass index (BMI) was calculated as weight divided by the square of height in meters.

Waist circumference was measured at the midpoint between the lower margin of the least palpable rib and the top of the hip or minimal waist using stretch-resistant tape while the subject stand with feet closes together thereby body weight evenly distributed arms at the side and wearing light clothing. When the subject became at a relaxed state measurement was taken at the end of normal expiration and this measurement was done in a private place (100).

4.6.3 Blood Pressure Measurement

Blood pressure was measured digitally (Micro life BP A50, Micro life AG, Switzerland) from the left arm of each subject with a standard adult arm cuff of mercury-based sphygmomanometer(101). The measurement was made by nurses who were working at a

chronic illness-follow up clinic after patients rested for at least 10 minutes. After each blood pressure measurement sphygmomanometer was cleaned with 70% alcohol.

4.7 Specimen Collection, Processing and Biochemical Tests

Five milliliters of venous blood sample was collected from study participants after overnight fasting using an aseptic technique and centrifuged after 30 minutes at 3000rpm, for 10 minutes. Separated serum was kept at -20°C in the refrigerator till used. The serum level of glucose, TC, HDL-c, LDL-c, TG, and cardiac troponin-I were measured using ARCHITECT c4000 high-sensitive troponin-I assay (Abbot Laboratories, Abbot Park, IL, USA) random access full automated auto analyzer (See Annex).

4.8. Quality Assurance

The questionnaire was initially prepared in English then; it was translated into local languages (both Afaan Oromoo and Amharic) by experts. One day of training on the contents of the questionnaire, data collection techniques, and research ethics was given for data collectors. Pretest of the questionnaire was conducted in 5% of subjects at Shanan Gibe Hospital before actual data collection and some adjustment on additional preparations was made. During the actual data collection period, the questionnaire was checked for completeness every night after data collection.

Data quality was also assured during blood sample collection by strictly following the standard aseptic operational procedure. The kit was made free from contamination and check for consistency. Laboratory analysis was done following the appropriate procedures based on the manufacturer's instruction. All the laboratory procedures were handled with the assistance of professional laboratory technologists and results were checked for completeness by the supervisors to maintain the overall quality of data. During data entry and analysis using computer software, due attention was given to keep the data quality.

4.9. Data Management and Analysis

All data were checked, cleared, and fed into Epi-data (version 3.1) and then exported to SPSS (version 25.0) software for statistical analysis. After the complete entry of all the data, a soft copy was checked with its hard copy to see the consistency. The data were also checked; for the fulfillment of assumption. It was processed by using descriptive analysis, including frequency distribution. The association of independent variables with cardiac

troponin-I was carried out using binary logistic regression. All independent variables with a p-value <0.2 in the bivariate logistic analysis were fitted into a multivariable logistic regression to identify independently associated factors in the final model. The degree of association was interpreted by using ORs with 95% CI and P <0.05 was considered as statistically significant. The Hosmer-Lemeshow test was used to check the appropriateness of the model for analysis .Comparison of mean difference of serum cardiac troponin-I among different types of anti-diabetic drugs was done using one way ANOVA after checking its assumption.

4.10 Ethical Consideration

Before starting the research ethical review committee of Jimma University approved this research project and a letter of ethical clearance was obtained with protocol Number IHRPGD 715/20. Then formal letter was obtained from Jimma university institution of health and taken to the chief clinical director of JMC and the permission letter was offered.

Next, the permission letter was given to the chronic follow-up clinic to conduct the study. To prevent COVID-19, data collectors used personal protective equipment like medical masks, disposable gloves, and hand sanitizer. Additionally, the distance between data collectors and study participants was kept at least 2m apart during face to face interview. Study participants were also encouraged to use a face mask and hand sanitizer as per the national protocol of COVID-19 prevention strategies. During data collection, only one participant was allowed in a room at a time.

After getting permission from the study participant, an information sheet with a detailed explanation of objectives, risks, and benefits to the study subject and the confidentiality of responses was given to participants. All study participants were informed about the research; the right to withdraw themselves at any time and confidentiality of information to be maintained during data collection, analysis, interpretation, and publication of result. The written consent was granted from each study participants based on their interest.

The confidentiality of the data was insured by using code rather than patients name and all the data results that showed deviation from normal reference was reported to physicians working in chronic disease follow up clinic.

4.11. Operational Definitions

Physical activity: any bodily movement produced by skeletal muscles that requires energy expenditure – including activities undertaken while working, playing, carrying out household chores, travelling, and engaging in recreational pursuits. Subjects who are engaged in leisure time physical activity (walking, fitness training and sports) for greater than or equal to three times per week of thirty minutes per occasion will be classified as physically active(102).

Body mass index (BMI): defined as the weight in kilograms divided by the square of height in a meter (kg/m2) is used to measure the degree of fatness. Normal weight is characterized by a BMI of between 18.5 and 24.9 kg/m2. Overweight is defined as BMI values between 25 and 29.9kg/m2 .Obesity is defined as a condition of abnormal or excessive fat accumulation in the adipose tissue of the body which is explained by BMI value > 30kg/m2(103).

Elevated blood pressure -defined as a systolic blood pressure (SBP) of ≥140 mm Hg or a diastolic blood pressure (DBP) ≥90 mm Hg(104).

Controlled blood sugar- Fasting blood sugar 80-130 mg/dl(105).

Patients on Anti-diabetic drugs: Patients who were taking any one of the following drugs at least for one year: insulin, metformin, insulin and glibenclamide, metformin and glibenclamide

Elevated cardiac troponin: Elevated cardiac troponin was considered as a serum cardiac troponinI level >34.2 pg/ml for male and >15.6 pg/ml for female at 99^{th} percentile upper reference limit.

Increased waist circumference: Defined as waist circumference ≥ 102cm for male and 88cm for female (100).

4.12 Dissemination Plan

The finding of this study will be submitted to department of Biomedical Sciences and School of Graduate Studies and presented to Jimma University community as a part of master's thesis. The copy of this stud will be submitted to JMC. It will also be published through publication on reputable journals and presented on scientific conference

5. RESULT

5.1 Socio-demographic Characteristics

A total of 82 type-2 diabetic patients on follow up were included in the study, of which 44(53.66 %) of them were males and the rest were females. Subjects included in the study were in the age group of 23 to 85 years old with a mean age of 53.41±13.85. The majority of them were greater than or equal to 60 years old 25(30.5%). Concerning educational status, 28(34.1%) completed primary education while only 10(12.2%) completed tertiary education. The majority of the study participants 27(32.9%) earn a monthly income of 1,000 to 1,999 Ethiopian birr (Table 1).

Table 1: Socio-demographic Characteristics of Adult Type-2 Diabetic Patients on Chronic follow up at JMC, Jimma, 2020

Variables		Frequency	Percentage (%)
Age[a]	<40	21	25.6
(Mean ±SD) 53.41± 13.85	40-50	16	19.5
	50-59	20	24.4
	≥60	25	30.5
Sex	Males	44	53.66
	Females	38	46.34
Educational status	No formal education	26	31.7
	Primary education	28	34.1
	Secondary education	18	22.0
	Tertiary education	10	12.2
Family history of DM	Yes	16	19.5
	No	66	80.5
Monthly income	<999	16	19.5
	1,000-1,999	27	32.9
	2,000-2,999	20	24.4
	≥3,000	19	23.2

5.2 Behavioral and Clinical Characteristics

Regarding behavioral characteristics of the study participants' majority of them were non-smokers 74(90.2%) and had no history of drinking alcohol 75(91.5%). Most of the respondents were physically inactive 57(69.5%). The mean duration since the diagnosis of diabetes mellitus was 6.6 ±5.87 years of which the majority of them 64(78%) had less than 10 years. Considering the type of anti-diabetic drugs, the majority 26(31.7%) were on insulin while 14(17.1%) were taking a combination of insulin and metformin (Table 2).

Table 2: Behavioral and Clinical Characteristics of Adult Type-2 Diabetic Patients on Chronic Follow up at JMC, Jimma, 2020

Variables		Frequency	Percentage (%)
Smoking	Yes	8	9.8
	No	74	90.2
Alcohol	Yes	7	8.5
	No	75	91.5
Physical activity	Yes	57	30.5
	No	25	69.5
Duration of DM	<10 years	64	78
	≥10years	18	22
Types of drug	Insulin	26	31.7
	Metformin	24	29.3
	Insulin + Metformin	14	17.1
	Metformin+ Glibenclamide	18	22.0

5.3 Anthropometric and Biochemical Characteristics

The mean±SD BMI of study participants was 25±4.48 kg/m^2, with 41(50.0%) were in the normal range. The mean ± SD blood pressure of study participants was 131.32±16.56 mmHg for SBP and 78.49±10.124 mmHg for DBP; with 27(32.9%)elevated SBP and 6(7.3%) elevated DBP. The mean ± SD waist circumference of the study participants was 93.93±13.55cm and 15(18.3%) of them had an elevated WC.

Regarding the lipid panel of respondents, the mean ± SD of cholesterol was 144.91±47.97 while that of LDL was 87.93±38.65. The Mean ± SD of TG and HDL were 117.33±53.29 and 32.99 ±9.78 respectively. The mean ± SD of cTnI was 17.896±9.976 (Table 3).

Table 3: Anthropometric and Biochemical Characteristics of Adult T2DM Patients on Chronic Follow up at JMC, Jimma, 2020

Variables		Category	Frequency (%)	Mean ±SD
Anthropometry	BMI(Kg/m^2)	Underweight	4(4.9)	25 ±4.48 kg/m^2
		Normal	41(50.0)	
		Overweight	25(20.5)	
		Obese	12(14.6)	
	WC(Cm)	Normal	67(81.7)	93.93±13.55 cm
		Increased	15(18.3)	
Blood pressure	SBP(mmHg)	Normal	55(67.1)	131.32±16.56 mmHg
		Increased	27(32.9)	
	DBP(mmHg)	Normal	76(92.7)	78.49±10.06 mmHg
		Increased	6(7.3)	
Blood glucose	FBS(mg/dl)	Normal	61(74.4)	155.05±60.01 mg/dl
		Increased	21(25.6)	
Lipid Panel(mg/dl)	TC	Normal	56(68.3)	144.91±47.97
		Increased	26(31.7)	
	LDL	Normal	57(69.5))	87.93±38.65
		Increased	25(30.5)	
	TG	Normal	56(68..3)	117.33±53.29
		Increased	26(31.7)	
	HDL	Normal	64(78.0)	32.99±9.78
		Increased	18(22.0)	

BMI-Body mass index, WC-Waist circumference, SBP-Systolic blood pressure DBP-Diastolic blood pressure, FBS-Fasting blood sugar, LDL-Low density lipoprotein, TG-Triacylglycerol, HDL- High density lipoprotein

5.4 Serum Levels of cTnI Mean±SD and Prevalence of Cardiac Troponin-I

The mean ± SD of cardiac troponin-I in study participants was 17.896 ± 9.796. It was highest in age group ≥60 years old (20.832 ± 11.587) but lowest in age group < 40 years old (15.905 ± 6.669). Regarding the serum level of cardiac troponin-I according to sex, the mean ± SD was higher in male than female (20.9 20.959 ± 10.848, 14.350 ± 7.012). Study participants with monthly income of ≥3000 ETB showed highest serum cTnI levels while those earned <999 ETB showed lowest serum cTnI (18.668±11.471 Vs 15.681± 9.496).

The overall prevalence of elevated cardiac troponin-I was 25.6% (21/82) among the study participants with the highest prevalence (14.63%) found in the age group ≥ 60 years old. The prevalence of cTnI was higher in males (14.63%) than females (10.97%).Concerning physical activity it was higher in physically inactive (23.17%) participants than active one (2.44%) (Table: 5)

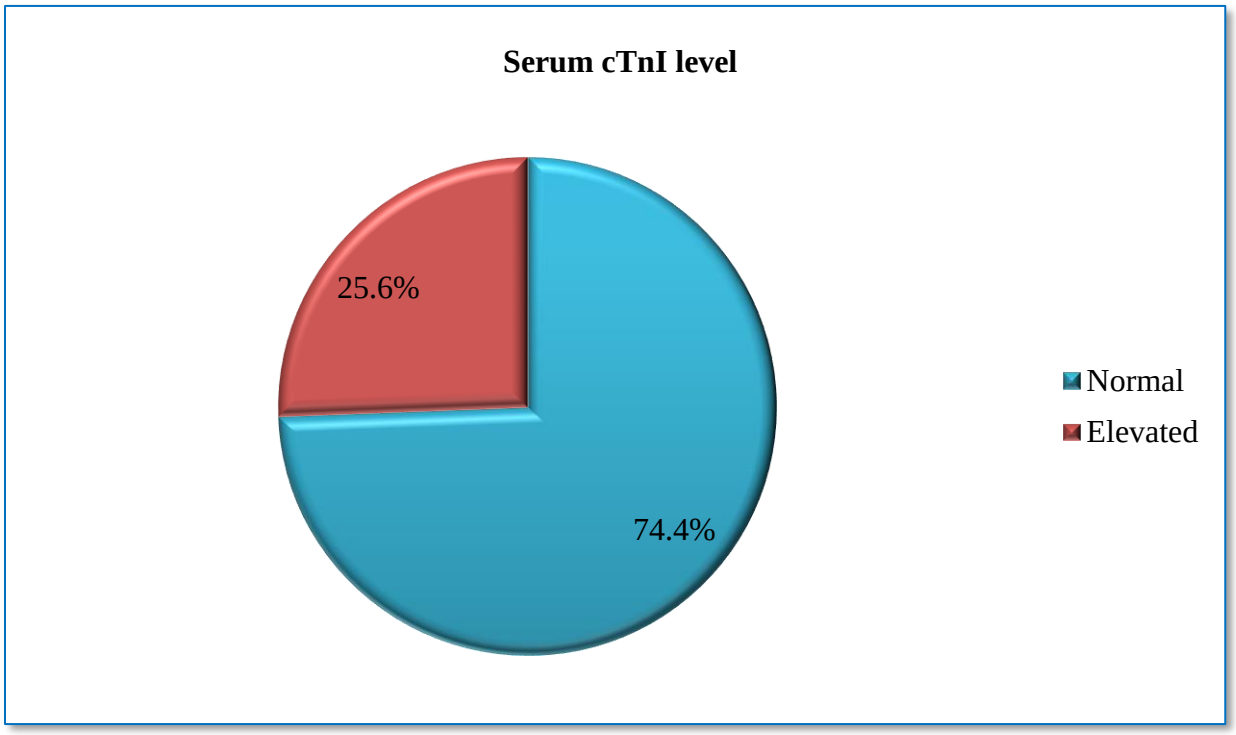

Figure 4: Prevalence of elevated cTnI among adult T2DM patients on chronic follow up at JMC, Jimma, 2020

5.5 Comparison of cTnI in Patients Taking Different Antidiabetic Medications

Patients who were taking metformin medication alone showed a significant mean decrease in serum cTn-I compared to the group on insulin alone(11.654 ± 1.6795 vs 20.573 ± 1.8402, P<0.01) also as compared to patients who were on metformin and glibenclamide combination, patients on metformin alone showed significant (21.094 ± 2.4062 vs11.654 1.6795, P<0.01) mean decrease in serum cardiac troponin-I. Patients receiving metformin alone also had numerically decreased in mean of cardiac troponin-I compared to individuals on a combination of insulin and metformin (11.654 ± 1.6795 vs 19.514 ± 2.2249) but it was not statistically significant. On the other hand, patients who were taking insulin and metformin combination showed a numerical decrease in cardiac troponin-I as compared to patients on insulin alone (19.524 ± 2.2249 vs 20.573 ± 1.8402) as well as on metformin and glibenclamide combination (19.514 ± 2.2249 vs 21.094 ± 2.4062) but statistically not significant (Fig.5 & Table 4)

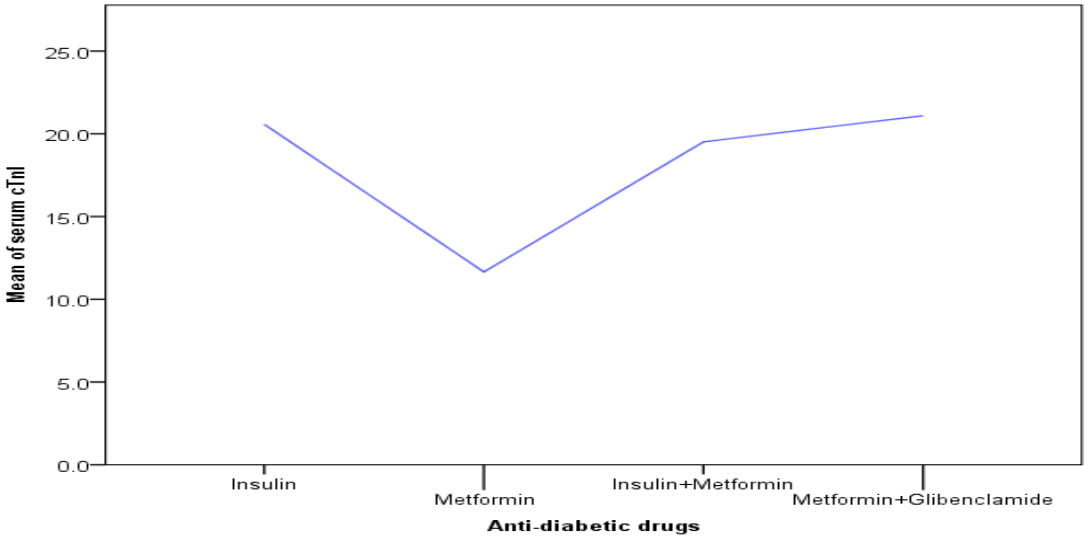

Figure 5: Mean of serum cTnI level among type-2 diabetic patients on different Anti-diabetic drugs at JMC, Jimma, 2020

After determining the presence of differences in mean value of serum cardiac troponin-I according to the type of Anti-diabetic drugs, the Tukey HSD post hoc analysis was conducted and showed that there was statistically significant difference in mean serum cardiac troponin-I between patients who were taking insulin monotherapy and metformin monotherapy also between patients who were on metformin monotherapy versus metformin and glibenclamide at P<0.05 (Table: 4)

Table 4: Tukey HSD Post hoc analysis for pairwise multiple Comparison of cTnI in patients on different types of anti-diabetic drugs

(I)Type of drug	(J) Type of drug	Mean Difference (I-J)	Std. Error	Sig.
Insulin	Metformin	8.9189*	2.5703	**.005**
	Insulin+Metformin	1.0588	1.0588	3.0100
	Metformin+glibemclamide	-.5214	-.5214	2.7841
Metformin	Insulin	-8.9189*	2.5703	**.005**
	Insulin+Metformin	-7.8601	3.0536	.057
	Metformin+glibemclamide	-9.4403*	2.8312	**.007**
Insulin+Metformin	Insulin	-1.0588	3.0100	.985
	Metformin	7.8601	3.0536	.057
	Metformin+glibemclamide	-1.5802	3.2356	.961
Metformin+glibemclamide	Insulin	.5214	2.7841	.998
	Metformin	9.4403*	2.8312	**.007**
	Insulin+Metformin	1.5802	3.2356	.961

*The mean difference is significant at the 0.05 level.

5.6 Factors Associated with Cardiac Troponin-I

5.6.1 Bivariate Logistic Regression Analysis of Socio- demographic, Behavioral and Clinical Risk Factors

From bivariate logistic regression analysis age, types of drug and physical activity were candidates for multivariable logistic regression analysis at $P < 0.2$ (Table 5)

Table5: Bivariate Logistic Regression Analysis of Cardiac Troponin-I by Sociodemographic, Behavioral and Clinical Risk Factors among Adult T2DM Patients on Chronic Follow up at JMC, Jimma, 2020.

Variables	Category	cTnI elevation		COR (95% CI)	P-Value
		No=61 N (%)	Yes=21 N (%)		
Age	<40	20(32.8)	1(4.8)	1	
	40-50	13(21.3)	3(14.3)	4.615(0.432-4.929)	0.206
	50-59	15(24.6)	5(23.8)	6.667(0.703-16.318)	**0.098**
	≥60	13(21.3)	12(57.1)	18.462(2.137-18.947)	**0.008**
Sex	Male	32(52.5)	12(57.1)	1.208(0.445-3.284)	0.711
	Female	29(47.5)	9(42.9)	1	
Educational status	No formal education	18(29.5)	8(38.1)	1.778(0.306-10.324)	0.521
	Primary education	21(34.4)	7(33.3)	1.333(0.227-7.827)	0.750
	Secondary education	14(23.0)	4(19.0)	1.143(0.170-7.693)	0.891
	Tertiary education	8(13.1)	2(9.5)	1	
Monthly income	<999	12(19.7)	4(19.0)	0.722(0.163-3.200)	0.668
	1000-1999	22(36.1)	5(23.8)	0.492(0.125-1.939)	0.311
	2000-2999	14(23.0)	6(28.6)	0.929(0.238-3.619)	0.915
	≥3000	13(21.3)	6(28.6)	1	
Smoking	Yes	5(8.2)	3(14.3)	1.867(0.406- 8.592)	0.423
	No	56(91.8)	18(85.7)	1	
Alcohol	Yes	5(8.2)	2(9.5)	1.179(0.211-6.587)	0.851
	No	56(91.8)	19(90.5)	1	
Physical activity	Active	23(37.7)	2(9.5)	1	
	Inactive	38(62.3)	19(90.5)	5.750(1.225-26.991)	**0.027**
Duration of DM	<10yrs	49(80.3)	15(71.4)	1	
	≥10yrs	12(19.7)	6(28.6)	1.633(0.523-5.096)	0.398
Antidiabetic drugs	Insulin	19(31.1)	7(33.3)	0.295(0.083-1.051)	**0.060**
	Metformin	22(36.1)	2(9.5)	0.073(0.013-0.406)	**0.003**
	Insulin+Metformin	12(19.7)	2(9.5)	0.133(0.023-0.777)	**0.025**
	Metformin+ glibenclamide	8(13.1)	10(47.6)	1	

5.6.2 Bivariate Logistic Regression Analysis of Anthropometric, Blood Pressure and Biochemical Parameters

Using bivariate logistic regression analysis SBP, LDL, total cholesterol, TG and waist circumference became candidates for multivariable logistic regression analysis at P< 0.2 (Table 6)

Table 6: Bivariate Logistic regression Analysis of cTnI by Anthropometric, Blood pressure and Biochemical Parameters among Adult T2DM Patients on Chronic Follow up at JMC, Jimma, 2020

Parameters	Category	Elevated cTnI		COR(95% CI)	P-Value
		No=61	Yes=21		
		N (%)	N (%)		
BMI	Underweight	3(4.9)	1(4.8)	1.185(0.110-12.818)	0.889
	Normal	32(52.5)	9(42.9)	1	
	Overweight	18(29.5)	7(33.3)	1.383(0.440-4.341)	0.579
	Obese	8(13.1)	4(19.0)	1.778(0.434-7.280)	0.424
WC	Normal	53(86.9)	14(66.7)	1	
	Increased	8(13.1)	7(33.3)	3.312(1.025-10.704)	**0.045**
SBP	Normal	44(72.1)	11(52.4)	1	
	Increased	17(27.9)	10(47.6)	2.353(0.846-6.545)	**0.101**
DBP	Normal	57(93.4)	19(90.5)	1	
	Increased	4(6.6)	2(9.5)	1.500(0.254-8.851)	0.654
FBS	Normal	47(77)	14(66.7)	1	
	Increased	14(23)	7(33.3)	1.679(0.567-4.972)	0.350
LDL	Normal	51(83.6)	6(28.6)	1	
	Increased	10(14.6)	15(71.4)	12.750(3.979-40.851)	**0.000**
HDL	Normal	47(77)	17(81)	1	
	Decreased	14(23)	4(19)	0.790(0.228-2.735)	0.710
TG	Normal	48(78.7)	8(38.1)	1	
	Increased	13(21.3)	13(61.9)	6.000(2.052-17.544)	**0.001**
TC	Normal	49(80.3)	7(33.3)	1	
	Increased	12(19.7)	14(66.7)	8.167(2.704-24.664)	**0.000**

BMI-Body mass index, Waist circumference, SBP- Systolic blood pressure, DBP- Diastolic blood pressure, FBS- Fasting blood sugar, LDL- low density lipoprotein, HDL- High density lipoprotein, TG-Triacylglycerol, TC-Total cholesterol, 1=indicates reference category, COR= Crude odds ratio

5.6.3 Bivariate and Multivariable Logistic Regression Analysis of Factors Associated with Elevated Cardiac Troponin-I

From multivariable logistic regression age, SBP, TC, LDL-C, and TG had a positive association with elevated cardiac troponin-I. Metformin drug treatment was negatively associated with cardiac troponinI at P <0.05.

The older age group age (≥60 years old) was 14 times more likely (AOR=13.735, P< 0.05) to have elevated cardiac troponin-I compared to the age group < 40 years old. Subjects with systolic hypertension were more likely (AOR=2.004, P < 0.05) to have elevated cardiac troponin-I than normotensive individuals. Those participants who were on metformin monotherapy treatment were less likely (AOR= 0.015, P< 0.05) to have elevated cardiac troponin-I than those who were on insulin plus glibenclamide. Compared with normal, participants with higher cholesterol were 6 times more likely (AOR=6.022, P<0.05) to develop elevated cardiac troponin-I. Increased LDL was another important risk factor associated with elevated cardiac troponin-I. Those who had higher LDL were 2 times more likely hood to develop elevated cardiac troponin-I than those who didn't have (AOR=2.146, P<0.05). Additionally, individuals with increased TG were also 2 times more likely to have elevated cardiac troponin compared to normal (AOR=2.468, P< 0.05) Table 7).

Table 7: Bivariate and Multivariable Logistic Regression Analysis of Factors Associated with Elevated Cardiac Troponin-I among Adult Type -2 Diabetic Patients on Chronic Follow up at JMC, Jimma, 2020

Variable	Category	COR (95%CI)	AOR (95%CI)	P-Value
Age	<40	1	1	
	40-50	4.615	5.687(0.050-6.462)	0.472
	50-59	6.667	8.925(0.281-12.833)	0.215
	≥60	18.462	13.735(2.849-16.622)	**0.013***
Physical activity	Active	1	1	
	Inactive	5.750	0.765(0.011-5.2880	0.902
SBP	Normal	1	1	
	Increased	2.353	2.004((2.000-4.455)	**0.022***
Types of drug	Insulin	0.295	0.076(0.004-1.309)	0.076
	Metformin	0.073	0.015(0.001-0.435)	**0.015***
	Insulin +Metformin	0.133	0.042(0.000-5.906)	0.209
	Metformin+ Glibenclamide	1	1	
WC	Normal	1	1	
	Increased	3.312	2.524(0.515-12.360)	0.104
TC	Normal	1	1	
	Increased	8.167	6.022(1.225-12.961)	**0.039***
LDL	Normal	1	1	
	Increased	12.750	2.416(1.744-3.346)	**0.018***
TG	Normal	1	1	
	Increased	6.000	2.468(1.032-5.903)	**0.048***

SBP- Systolic Blood Pressure, WC- Waist circumference, LDL- Low Density Lipoprotein, TG- Triacylglycerol. *P significant at <0.05, 1=Reference Category, COR=Crude odds ratio, AOR= Adjusted Odds ratio

6. DISCUSSION

Diabetic patients often exhibit silent cardiac dysfunction which is detectable only in the latter stage of the disease. Even ≈50% of individuals with well-controlled diabetes mellitus, asymptomatic and normotensive patients are considered to exhibit some degree of cardiac dysfunction(35). High sensitive cardiac markers such as cardiac troponin-I is currently considered as the gold standard diagnostic tool of myocardial injury(10).

In this study, 25.6% (21/82) of the study participants showed elevated cardiac troponin-I. This was in line with a study conducted in the USA where cardiac troponin was elevated in 26% of type-2 diabetic patients(97). But it was higher than the study in India that reported the prevalence of elevated cardiac troponin-I as 20.7% in acute srtoke patients(106) and the study conducted in the UK that reported the prevalence of elevated cardiac troponin-I(12.4%) among patients who were attending emergency departments without Acute coronary syndrome(107). On the other hand, the finding of this study was lower than the study in USA that reported 32.3% of elevated cardiac troponin-I in patients with hypertensive emergency(108). The discrepancy may be due to the difference in the study population, sample size and study design.

This study also found that older age was significantly associated with elevated cardiac troponin-I. Patients greater than or equal to 60 years old were 14 times (AOR=13.75 95% CI, P= 0.013) more likely to develop elevated cardiac troponin-I compared to individuals under 40 years old. This finding was in agreement with studies done in Boston, UK and Switzerland where the older age was positively associated with higher Cardiac troponin-I(95,107–109). Study in Turkey also found elevation of cardiac troponin-I in persons above 40 years old compared to younger age(110). This may be due to aging-induced structural, functional, cellular, and molecular changes responsible for a decrease in the adaptive capacity of the cardiac cell and loss of cardiomyocytes(111). From a structural change point perspective, cardiac aging is associated with left ventricular hypertrophy, fibrosis, and diastolic dysfunction. Cardiomyocytes apoptosis and vascular stiffness are also attributable to aging-induced structural and functional modifications(111,112). Age-dependent decline in mitochondrial function and accumulation of senescent cells may also lead to an increase in cell death(113).

High systolic blood pressure was significantly associated with elevated cardiac troponin in this study. A person with high systolic blood pressure was more likely to have elevated cardiac troponin-I compared to normal systolic blood pressure (AOR=2.004, p=0.022). In agreement with our finding Stefanie *et al.* and Bossard *et al.* reported a positive association of cardiac troponin-I and systolic blood pressure(109,114). This is probably due to endothelial dysfunction related to an increased level of Renin-angiotensin dysregulation which leads to increased Angiotensin II, decreased nitric oxide production, and increased oxidative stress all of which ultimately cause inflammation and thrombotic complications (115). Endothelial dysfunction is also associated with increased arterial stiffness, leading to cardiac hypertrophy and myocardial ischemia(116).

In contrary to the current study David *et al.* found that elevated cardiac troponin was associated with normal or lower systolic blood pressure. The discrepancy of this study result may be due to genetic and environmental variation of the study participants (117).

This study also found that hypercholesterolemia, hypertriglyceridemia, and LDL-C were another independent predictor for high cardiac troponin-I after multivariable logistic regression. This was consistent with a study conducted in Nepal that reported patients with chest pain and positive troponin test were found to have significantly elevated levels of total cholesterol, triacylglycerol levels, and low-density lipoprotein level(93).

It was also similar to a study done by Shivananda *et al.* and Afonso *et al.* which found a positive correlation of elevated cardiac troponin with total cholesterol, triglycerides, LDL, and a negative correlation with HDL-C (94,108). The effect of hypercholesterolemia on elevated cardiac troponin may be explained by cholesterol-induced atherosclerosis. A buildup of cholesterol and plaques on the inner walls of the coronary artery restricts blood flow to the heart muscle by physically clogging the artery or by causing abnormal artery tone and function that will exacerbate ischemic necrosis(118). LDL-C is the major contributor to plaque formation(119).

On the other hand, hypercholesterolemia may make the myocardium more sensitive to exogenous damage such as hemodynamic overload, myocardium ischemia, and diabetes. This is through the coordinated action of alteration of a membrane lipid bilayer, the regulation of intracellular calcium ions, and isoform expression patterns of myosin heavy

chain(120). Accumulation of total cholesterol in heart tissue decreases pgc-1 mRNA levels and reduces intracellular energy metabolism via aggravating UCP2 expression. Adverse effects on cardiac function are also associated with increased expression of peroxisome proliferator-activated receptor γ (PPARγ)(121). Excessive expression of PPAR may induce dilated cardiomyopathy, due to increased lipid storage and changes in mitochondrial structure(122). Hypertriglyceridemia may be linked to atherosclerosis through promoting the formation of foam cells, lipid exchange, vascular endothelial dysfunction, inflammatory response, and coagulation all of which enhance myocardial damage along with troponin release(123).

In contrary to this finding Giuseppe L *et al.* reported no association of LDL-C, TC and TG with cardiac troponin-I in patients attending inpatient clinics for cardiovascular risk assessment(124). This difference may be due to the difference in measurement method we used and the diversified nature of the study population

Multivariable logistic regression analysis also revealed that type-2 diabetic patients on metformin were significantly associated with decreased cardiac troponin-I (AOR= 0.015, $P<0.05$) compared to patients who were taking metformin plus glibenclamide (Table: 7).

The study conducted by Arif and Al- Ezzi on an animal model found a similar result; significant reduction of serum level of troponin-I in the metformin treated group(125). Another study conducted in Argentina reported lower cardiac troponin in patients who were taking metformin as well as glibenclamide compared to control. This finding was also consistent with our study regarding metformin(88). Concerning glibenclamide, our study was inconsistent with this finding the reason may be explained by the fact that our study was a comparison of metformin with glibenclamide containing treatment category, not with control a group. A similar result regarding the negative association of metformin with cardiac troponin was reported by Lexis *et al.*(90). Our finding was also supported by the study that compared the effect of metformin versus sulfonylurea/insulin treatments on a 10-year follow up period which found 33% reduction in myocardial infarction risk in contrary to 15% decrease by the latter(126). A systematic and meta-analysis study also revealed that metformin was effective in reducing the incidence of cardiovascular events compared to sulfonylureas(127).

Metformin may reduce cardiac troponin-I through several mechanisms, but the exact mechanism remains unclear(128). Metformin phosphorylates AMP- activated kinase which is related to many targets related to reduction the of I/R injury, and this activates Reperfusion Injury Salvage Kinase (RISK) path including PI3K and AKt pathways, increasing tumor suppressor gene p53, inactivation of mammalian target of rapamycin (mTOR) and activation of endothelial nitric oxide synthase (eNOS)(129).

The other mechanism of action of metformin may be through enhancing glucose utilization of the heart by increasing glucose transporters (GLUT-1 and GLUT-4). Moreover, metformin activates the adenosine receptor via increased intracellular formation of adenosine, possibly reducing MI size (130). On the other hand, metformin is associated with a decrease in Dipeptidyl peptidase-4 activity and an increase in circulating levels of glucagon-like peptide-1, which are associated with a reduction of MI size(91,131). Overall, these metformin-induced changes in myocardial gene and energy program, are due to the fact that the activation of AMPK (132,133). In addition metformin has also several beneficial effect such as anti-inflammatory benefits, reduces oxidative stress, lowers endothelial dysfunction , and reduces hypertension(126).

This finding did not agree with the study done by Basnet *et al.* that reported no significant association between metformin treatment and MI(91). This disagreement may be due to the evaluation of troponin in the latter study group was carried out in a population already developed myocardial infarction.

7. Limitation of the study

Although necessary efforts have been made to overcome the problem, it is inevitable that there are limitations, and the results should be interpreted taking the following limitations into consideration. First the result may not be generalized to the entire population of type-2 diabetic patients due to the small sample size for the matter of resource limitation. Next serial measurement of cardiac troponin-I at different time intervals was not done due to budget constraints. Additionally, comparative non-diabetic patients were not included in the study due to budget limitations. Eventually, causal inferences or temporal associations were not determined because of cross sectional nature of study design.

8. CONCLUSION AND RECOMMENDATIONS

8.1 Conclusion

This study found that about one fourth of type-2 diabetic patients on follow up had elevated cardiac troponin-I. Older age (≥60years), systolic blood pressure and dyslipidemia were predictors of an elevated cardiac troponin-I. Metformin significantly reduced serum cTnI levels compared to insulin monotherapy and metformin + glibenclamide.

7.1 Recommendations

- Medical staffs need to regularly assess cardiac status of T2DM patients using high sensitivity cardiac markers such as troponin-I and create awareness toward factors associated with diabetic heart disease during follow up.
- Researchers need to conduct studies that include multiple centers and large sample sizes.
- Researchers also need to conduct studies that compare cardiovascular effect of different anti-diabetic drugs using longitudinal and cohort study design in the Ethiopian population.
- Health policy makers have to work on prevention of non-communicable disease and risk reduction of diabetes mellitus related vascular complications.

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ANNEXES

Annex I: Participant's Information sheet (English Version)

Principal Investigator:- Alemayehu Babusha Wega

Dear participant! Here, I the undersigned, at Jimma University, Institution of Health, Faculty of medical Sciences department of Biomedical Sciences graduate Study Program, currently undertaking a research on a topic entitled as, Assessment of Cardiac Troponin-I and Associated Factors in Type-2 Diabetic Patients on Anti-Diabetic Drug treatment at Jimma Medical Centre, Jimma, Southwest Ethiopia. For this study, you will be selected as a participant and before getting your consent, you need to know all necessary information related to the study whose detail is explained as follows.

Objective: The objective of this study is to Assess Cardiac Troponin-I and Associated Factors in Type-2 Diabetic Patients on Anti-diabetic Drug treatment at Jimma Medical Center

Risks and discomfort: Participation in this study will not cause more discomfort and no need of extra sample other than sample taken for diagnostic purpose. But, there could be minor pain and challenge in color of your skin following the blood drawing. The amount of blood taken from each volunteer throughout the study period is 5ml which will not affect your health. We don't expect major risk in participating in this research, as the whole procedure is carried out by trained health professionals, in case any injury occurs suddenly you will be treated by principal investigator's expense.

Benefits:-There is no immediate benefit from participating in this study. However, you will have the chance to know your serum cardiac troponin level, lipid profile, Blood pressure, and BMI measurements. Moreover, if your result reveals any incidental health problems that need immediate treatment, we will make an arrangement the way you get first chance to contact concerned physician as well as referral facility if needed. In addition, your participation will contribute in improving the health delivery system for T2DM patients.

Confidentiality: The information which is collected from you will be kept confidential and used only for research purpose. We assign a code to questionnaires and blood sample to assure confidentiality instead of using your name and we don't disclose the information to third body.

Participant's Right : Your participation is voluntary and you are not obliged to answer any question you do not want to answer. If you are not comfortable with interview you can leave at any time you need as there is no harm if you don't answer the questions.

Persons to contact: For more information and question here is the contact address of investigator: Alemayehu Babusha; Tel: 0917666429; email: Alemayehubabusha@gmail.com

Participant's consent form English Version

I have been informed the importance and Objective of the study going to be conducted by Mr. Alemayehu Babusha on the Assessment of Cardiac Troponin-I and Associated factors in Type-2 diabetic patients on Anti-diabetic Treatment at Jimma Medical Center, Jimma, Southwest Ethiopia. I had the chance to ask questions about the study and all questions have been answered to my understanding. I have been informed and understood that participation is entirely voluntary and that I can withdraw my consent at any time if I wish so. I consent voluntarily to participate in this study as a respondent.

Participant's Signature _____

Name of Data collector_____ Signature_____

Date: _____

Participants Information Sheet (Amharic Version)

የተሳታፊዎች መረጃ ቅፅ

የጥናቱ ርህስ፡ በጅማ የህክምና ማህከል የሁለተኛ ሀይነት የስኪር በሽታ መድሓኒት የምወስዱትን በሽተኞች የልብ ትሮፖኒን-I እና ተያያዥ ነገሮችን መገምገም ይሆናል።

እኔ አለማየሁ ባቡሻ በጅማ ዪኒቨርሲቲ ጤና ተቋም ሜዲካል ፋካልቲ በባዮሜዲካል ትምርት ክፍል የድህረ ምረቃ ተማሪ ስሆን የመመረቅያ ጽሁፌን በጅማ የህክምና ማህከል የሁለተኛ ሀይነት የስኪር በሽታ መድሓኒት የምወስዱትን በሽተኞች የልብ ትሮፖኒን-I እና ተያያዥ ነገሮችን መገምገም በምል ርህስ በመስራት ላይ ነኝ። እርሶም ለዚህ ጥናት የተመረጡ ስለሆነ ከዚህ ቀጥሎ የምገኘዉን መረጃ አንብበዉ ጥናቱ ላይ ለመሳተፍ መስማማትዎን ወይም አለመሰማማትዎን እንድያረጋግጡ በትህትና እጠይቃለሁ።

መግቢያ፡ ጥናቱ ከእርሶ የሚወስዳቸዉ ማንኛዉም መረጃዎች ምስጥራዊነት ሙሉበሙሉ የተጠበቀ ሲሆን እርሶ ጥናቱ አለመሳተፍም ሆነ በማንኛዉም ሰአት ተሳትፎዎን ማቋረጥ ይችላሉ።

የጥናቱ አላማ፡- የጥናቱ ዋና አላማ በጅማ የህክምና ማህከል የሁለተኛ ሀይነት የስኪር በሽታ መድሓኒት የምወስዱትን በሽተኞች የልብ ትሮፖኒን እና ተያያዥ ነገሮችን መገምገም ይሆናል። የጥናቱ ዉጤት ለስኪር ህሙማን ጤና እንክብካቤ የሚጠቅም ሲሆን ከዚህም በተጨማሪ እርስዎም ከላይ የተጠቀሱትን ነገሮች እንዲያዉቁ ይረዳዎታል። በጥናቱ ላይ የሚያደርጉት ተሳትፎ ሙሉ በሙሉ በእርሶ በጎ ፈቃደኝነት ላይ የተመሰረተ ነዉ።

ጉዳት፡-በጥናቱ ለመሳተፍ ፍቃደኛ ከሆኑ ለናሙና ይሆን ዘንድ አምስት ሚሊሊትር ያህል ደም በሆስፒታሉ የጤና ባለሙያዎች አማካኝነት የሚሰጡ ሲሆን ናሙና በሚሰጡበት ጊዜ ሁልጊዜ ለምርመራ ከሚሰጡት የተለየ የህመም እና አለመመቸት የለዉም። የደም ናሙና የምወስዱት የሠለጠኑ ጤና ባለሙያዎች ከመሆኑ አንጻር ብዙም የጤና ጉዳትያመጣል ብለን አንጠብቅም፤ይሁን እነጂ እንደ አጋጣሚ ሆኖ ችግር ከተፈጠረ በእኛ ወጪ የምናሳክምዎ መሆኑን እናሳዉቆታለን።

ጥቅም፡- በዚህ ጥናት በመሳተፍዎ በቀጥታ የምያገኙት ጥቅማጥቅም አይኖርም፤ይሁን እነጂ የልብዎን የትሮፖኒን መጠን፡ የደም ግፊት ልኬትና ክብደቶን ለማወቅ ይጠቅሞታል። ከዚህ ጋር ተያይዞ ችግር ከተገኝብዎት ከምመለከተዉ ዶ/ር ጋር እናገናኝዎታለን፤ችግርዎ ወደሌላ ጤናተቋም የሚያስልክ ሆኖ ከተገኘ የምላኩበትን ሁኔታ እናመቻችሎታለን።

ምስጥርነት፡ ከእርሶ የምሰበሰብ መረጃ ምስጥርነቱ የተጠበቀ እና ለጥናቱ ሀላማ ብቻ የምዉል መሆኑን ከወዲዉ እናሳዉቆታለን። ምስጥርነት መጠበቁን የምናረጋግጥልዎ ደግሞ ስምዎን ሳይሆን የምስጥር ቁጥር በመጠቀማችን ነዉ፤ በተጨማሪም መረጃዉን ለሶሰተኛ አካልአሳልፈን የማንሰጥ መሆኑን እናረጋግጣለን።

ከጥናቱ ጋር በተያያዘ ጥያቄ ቢኖርዎት ወይም ችግር ቢያጋጥሞ በማንኛዉም ሰአት በሞባይል ቁጥር 0917666429 አለማየሁ ባቡሻ ብለዉ ይደዉሉ ወይም በኢሜል አድራሻ Alemayehubabusha@gmail.com መላክ ይችላሉ።

በጥናቱ ለመሳተፍ ፍቃደኛ ከሆኑ እባክዎ ከዚህ ቀጥሎ ባለዉ የስምምነት ቅፅ ላይ በመፈረም ይተባበሩን።

ለትብብርዎ እናመሰግናለን

Informed consent form (Amharic Version)

የፈቃደኝነት ማረጋገጫ ቅጽ

በጅማ የህክምና ማህከል የሁለተኛ ሀይነት የስኪር በሽታ መድሓኒት የምወስዱትን በሽተኞች የልብ ትሮፖኒን-I እና ተያያዥ ነገሮችን መገምገም በምል ርህስ ላይ የምሰራ ምርምር አስፈላግነቱ እና ዐላመዉ ተብራረቶልኛል። እኔም በተብራራልኝ መንገድ ተረድቻለሁ። ምርምሩምንም የተለየየ ገንዘብ ወይም ጥቅማጥቅም የሌለዉ፣ አደጋ የማያስከትል መሆኑን እንዲሁም የሚደረገው ተሳትፎ እናመረጃ በሚስጢር የሚያዝና ለማንም ተላልፎ የማይሰጥ መሆኑን ተረድቻለሁ።

ስለዚህ በዚህ የምርምር ጥናት ላይ ለመሳተፍ ፈቃደኛ መሆኔን በፊርማዬአረጋግጣለሁ።

የተሳታፊው ፊርማ ---------- የመረጃ ሰብሳቢው ስም---------- ፊርማ-------

ቀን ----------

Participant's information sheet (Afan Oromo version)

Unka Odeeffannoo Hirmaattota Qorannichaa

Mata duree Qorannoo:- Giddu gala fayyaa Jimmaatti dhukkabsattoota Qoricha dhibee sukkaaraa gosa lammaffaa fudhatan irratti Tirooppooninii-I fi wantoota ittiin wal-qabatan qorachuu kan jedhu ta'a.

Ani maqaan kiyyaa Alamaayyoo Baabushaa kanan jedhamu barataa digirii lammaffaaJimmaa yuuniversitii dhaabbata fayyaa faakaaltii Saayinsii meedikaalaatti dippaartimeenti Saayinsii Baayoo-medikaalaa yommuun ta'uu, waraqaa qorannoo eebbaa mata dureen isaa Giddu gala fayyaa Jimmaatti dhukkabsattoota Qoricha dhibee sukkaaraa gosa lammaffaa fudhatan irratti Tirooppooniinii-I fi wantoota ittiin wal-qabatan qorachuu kan jedhu irrattan hojjechaa jira. Isinis dhimmuma kanaaf waan filatamtaniif odeeffannoo armaan gadii dubbisuudhaan qorannoo kana irratti hirmaachuu fi dhiisuu keessan akka nuuf mikaneessitan kabajaanin isin gaafadha.

Seensa: Odeeffannoon qorannoo kanaaf jecha isin irraa fuudhamu iccitiin isaa guutummaan guutuutti Kan eegamu fi qorannoo kana qofaaf Kan oolu yommuu ta'uu, isinis qorannoo irratti hirmaachuufi dhiisuu akka dandeessan akkasumas sa'aatii barbaaddanitti adda kuttanii ba'uu akka dandeessan gamanumaan isin beeksifna.

Miidhaa: Qorannoo kana irratti hirmaachuuf fedha yoo qabaattan, sammuudaaf Kan ta'u dhiigi miililiitirii Shan (5ml) karaa ogeessa laaboraatooriitiin kan isin irraa fuudhamu yommuu ta'u kunis kan yeroo biraa qorannoof fuudhamuun ala dhukkubbii ykn miira biraa isin irratti uumu hinqabu. Sammuudni kan isin irraa fuudhamu ogeessota fayyaa leenjii qabaniin waanta'eef miidhaa fayyaa cimaa isin irran ga'a jennee hin eegnu. Hata'u malee akka tasaa yeroo Sammuudni fuudhamu miidhaan yoo isin irra ga'e baasii keenyaan kan isin yaalchisnu ta'uu gamanumaan isin beeksifna.

Faayidaa: Qorannoo kana irratti hirmaachuu keessaniif faayidaan isin kallattiin argattan hinjiru; hata'u malee agarsiistota fayyaa onnee keessanii, dhiibbaa dhiiga keessanii fi ulfaatina qaama keessanii fi kan ittiin wal-qabatan beekuuf isin gargaara. Akkasumas kanaan kan wal-qabatan rakkoon fayyaa yoo isin irratti mul'ate qaama dhimmi isaa ilaallatu waliin walisin quunnamsiifna.

Iccitii: Odeeffannoon isin irraa fuudhamu iccitiin isaa guutummaan guutuutti Kan eegamuufi dhimma qorannoof qofa kan ooluudha. Iccitiin Kun eegamu isaa kan ittiin mirkaneessinus

maqaa keessan utuu hinta'in Kooddii fayyadamuu keenyaani.Dabalataanis odeeffannoon isin irraa fuudhamu kun qorannoo qofaaf kan ooluufi qaama sadaffaaf dabarfameee kan hinkennamne ta'uu isaa nimirkaneessina.

Dhimma qorannoo kanaan walqabatee gaaffii ykn rakkoon yoo isin mudate lakkoofsa bilbilaa 0917666429 irratti Alamaayyoo Baabushaa jettanii bilbiluu nidandeessu.Dabalataanis Email- Alemayehubabusha@gmail.com jedhu irratti naquunnamuu dandeessu.

Qorannoo kana irratti hirmaachuuf fedha yoo qabaattan unka waliigaltee armaan gadii irratti mallatteessuun nuuf mirkaneessaa!

DEGGARSA KEESSANIIF GALATOOMA

Unka fedhii waliigaltee Afaan Oromootiin (Afan Oromo version)

Giddu gala fayyaa Jimmaatti dhukkabsattoota Qoricha dhibee sukkaaraa gosa lammaffaa fudhatan irratti Tirooppooninii-I fi wantoota ittiin wal-qabatan qorachuu kan jedhu irratti qorannoon adeemsifamu kaayyoo fi barbaachisumman isaa naaf ibsamee jira.Anis wanta naaf ibsamee hubadheen jira. Akkasumas sa'aatii barbaadetti hirmaannaakoo dhaabuu akkan danda'u natti himamee jira. Qorannoo kana irratti hirmaachuu kootiin faayidaa addaa (birrii) kanan hinarganne ta'uu, Miidhaa fayyaa cimaa kan narraan hin geenye akkasumas Odeeffannoon narraa fuudhamu iccitiin isaa kan eegamu fi qaama biraaf dabrfamee kan hin kennamne ta'uu hubadheen jira.

Kanaafuu Qorannoo kana irratti hirmaachuuf fedha qabaachuukoo Mallattoo kootiin nan mirkaneessaa.

Mallattoo isa/ishee hirmaattuu_____

Maqaa fi Mallattoo namicha odeeffannoo funaanuu_____

Annex II: Questionnaire (English version)

Participant's Registration number_____

Participant's code number_____

Date of interview: _____

Data collector's signature_____

Participant's signature_____ Tel.no_____

Part I: Socio-demographic Characteristics

1.1) Age in completed years_____

1.2) Sex A. Male B. Female

1.3) Educational status A. No formal education C. Secondary level (9-12)

B. primary level (1-8) D. Tertiary level (> 12)

1.4) Monthly income _____ETB

1.5) Do you have family history of diabetes? A. Yes B. No

II: Behavioral factors

2.1) Smoking status

A) Currently smoker, _____ cigarette per day

B) Quitted smoking, last smoked and number cigarette you were smoking per day before quitting _____

C) Never smoked D) missing information

2.2 If you smoked for how long? _____

2.3 Do you drink alcohol? A) Yes B) No

2.5 If you drink please fill the following table

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

2.6 Do you do physical activity? A) Yes B) NO

2.3 If yes on question number 2.6 above what physical activity you usually do? _____

2.4 If yes on question number 2.6 for how long you usually do? _____

2.5 If yes how often do you do in a week? _____

III Clinical factors

3.1 Do you have additional health problem other than diabetes? A. Yes B.NO

3.2 If yes for above question please specify the problem_____

3.3 Duration of diabetes _____

3.4 Duration since using the drug _____

3.5 Name of the drug being taken _____

3.6 Frequency of follow up_____

3.7 Weight in (Kg) _____

3.8 Height (cm) _____

3.9 Systolic arterial blood pressure (mmHg) _____

3.10 Diastolic arterial blood pressure (mmHg) _____

IV. Biochemical measurements

4.1 Fasting Blood sugar (FBS) _____

4.2 High density lipoprotein (HDL) _____

4.3 Low density lipoprotein (LDL) _____

4.4 Triacylglycerol (TG)_____

4.5 Total cholesterol (TC) _____

4.5 Cardiac troponin-I_____

Amharic version Questionnaire

የተጠያቂዉምዝገባቁጥር----------

የተጠያቂዉየምስጥርቁጥር---------- የተጠያቂዉስልክቁጥር ----------

የተጠያቂዉፊርማ ---------- የጠያቂዉ ፊርማ ---------- ቀን----------

ክፍል አንድ፡ የማህበረሰብ ና ስነ ህዝብ ባህሪያትን በተመለከተ

1) ህድሜ ----------

2) ጸታ ሀ) ወንድ ለ) ሴት

3) የትምህረት ደረጃ ሀ) ማንበብና መጻፍ የማይችል ለ) 1-8 ሐ)9-12 መ) ከ 12 በላይ

4) የወር ገቢዎ ---------- የእትዮጵያ ብር

5) ከቤተሰብዎ የስኪር በሽታ ያለበት ሰዉ አለ? ሀ) አዎ ለ) የለም

ክፍል ሁለት፡ ባህሪን በምመለከቱ ጥያቄዎች

2.1) ስጋራ ያጨሳሉ? ሀ) በፍጹም አጭሽ አላቅም ለ) አጨስ ነበር አሁን ግን አቁሜያለሁ ሐ) አዎ አጨሳለሁ

2.2) የሚያጨሱ ከሆነ ምን ያህል ጊዜ አጨሱ? ----------

2.3) በአማካይ በቀን ስንት ፓክ ስጋራ ያጨሳሉ? ----------

2.4) አልኮል ይጠጣሉ? ሀ) እጠጣለሁ ለ) አልጠጣም

2.5 የሚጠጡ ከሆነ እባክዎ የሚከተለውን ሠንጠረዥ ይሙሉት

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

2.6) የአካል ብቃት እንቅስቃሰ ይሰራሉ? ሀ) አዎ ለ) አልሰራም

2.7) የምሰሩ ከሆነ ምን ሀይነት የአካል ብቃት እንቅስቃሰ ነዉ የምሰሩት----------

2.8) በሳምንት ስንት ጊዜ ነዉ የምሰሩት ----------

2.9) በአንዴ ለምን ያህል ጊዜ ነዉ የምሰሩት (በሰአት ወይም በደቂቃ) ----------

ክፍል ሶስት፡ የጤና መረጃ

3.1) ከስኪር በሽታ ዉጭ ተጓዳኝ በሽታ አለብዎት? ሀ) አዎ ለ) የለብኝም

3.2) ካለብዎት የበሽታዉን ስም ይጥቀሱ ----------

3.3) የስኪር በሽታ እንዳለብዎት ካወቁ ምን ያህል ጊዜ ነዉ? ----------

3.4 ለስኪር በሽታ የምሰጥ መድሀኒት ከጀመሩ ምን ያህል ጊዜ ነዉ? ----------

3.5 የምወስዱትን መድሀኒት ስም----------

3.6 ክብደት በኪሎግራም----------

3.7 ቁመት በሳ.ሜትር ----------

3.8 የደም ግፊት ልኬት----------

ክፍል አረት፡ የላቦራቶሪ ምርመራ ዉጤት(በለቦራቶሪ ባለሙያ የምሞላ)

4.1 FBS _____

4.2 HDL _____

4.3 LDL _____

4.4 TG_____

4.5 Cardiac troponin I_____

Unka Gaaffilee Afaan Oromootiin

Lakkoofsa galmee/kaardii _____

Kooddii isa gaafatamuu/gaafatamtuu _____

Lakkoofsa bilbilaa isa/ishee gaafatamtuu_____

Mallattoo isa gaafatamuu_____

Mallattoo isa gaafatuu_____ Guyyaa_____

Kutaa I: Gaaffii dhimma hawaasummaa fi uummataa ilaalchisee

1.1) Umurii_____

1.2) Saala A) Dhiira B) Dhalaa

1.3) Sadarkaa barumsaa A)Barumsa idilee kan hinbaranne B)1-8 C)9-12 D)>12

1.4) Galii ji'aan birrii Itoophiyaatti yoo tilmaamamu_____

1.5) Maatii keessan keessaa namni dhukkuba sukkaaraa qabu jiraa? A)Jira B) hinjiru Kutaa

Kutaa II: Gaaffii dhimma amalaa irratti xiyyeeffatu

2.1) Tamboo nixuuxxuu? A) Xuuxee hinbeeku B) Xuuxaan ture amma dhiiseera C) Nan xuuxa

2.2) Kan xuuxxan yoo ta'e waggaa meeqa xuuxxan? _____

2.3) Guyyaatti paakii meeqa xuuxxu? _____

2.4) Alkoolii nidhugduu? A) Eeyyee B) Lakki

2.5) Nidhugdu yoo ta'e gabatee armaan gadii guutuun nugargaaraa

Gosa dhugaatii	Dhugaatii hanga dhugdan		
	Guyyaatti	Torbanitti	Ji'atti
Biiraa qaruuraadhaan			
Wayinii burcuqqoodhaan			
Dhugaatii nama macheessu safartuun			

2.6) Sochii qaamaa nihojjettuu? A) Eeyyee B) Lakki

2.7) Sochii qaamaa Kan hojjetten yoo ta'e sochii akkamiiti? _____

2.8) Torbanitti si'a meeqa hojjettu? _____

2.9) Si'a tokkicha daqiiqaa ykn sa'aatii meeqaaf hojjettu? _____

Kutaa III: Odeeffannoo waa'ee fayyaa

3.1) Dhukkuba sukkaaraan ala dhibee biraa qabduu? A) Eeyyee B) Lakki

3.2) Yoo qabaattan maqaa isaa caqasaa_____

3.3) Dhukkuba sukkaaraa qabaachuu keessan erga bartanii hangam ta'eera? _____

3.4) Qoricha dhibee sukkaaraa erga jalqabdanii hangam ta'a? _____

3.5) Maqaa qoricha fudhataa jirtanii_____

3.6 Ulfaatina kiiloogiraamaan_____

3.7 Hojjaa sentimeetiraan_____

3.8 Safara dhiibbaa dhiigaa_____

KutaaIV: Firii Qorannoo Laaboraatooriitiin (Ogeessa Laaboraatooriitiin kan guutamu)

4.1 FBS _____

4.2 HDL _____

4.3 LDL _____

4.4 TG_____

4.5 Cardiac troponin- I_____

Annex III: Laboratory Tests

Determination of cardiac troponin-I

It was determined by using ARCHITECTc4000 high sensitive troponin-I assay (Abbot Laboratories, Abbot Park, IL, USA) random access full automated autoanlyzer.

Test principle: Cardiac Troponin I Assay is based on a latex enhanced immunoturbidimetric assay. Cardiac Troponin-I in the sample binds to the specific anti-Troponin-I antibodies, which are coated on latex particles, and causes agglutination. The degree of turbidity caused by agglutination can be measured optically and is proportional to the amount of Troponin I in the sample.

The test kit recommends the elevated cTnI cut of value > 34.2pg/ml for males and >15.6pg/ml for females at 99th percentile upper reference limit.

4.7.3 Determination of lipid profile

4.7.3.1 Total Cholesterol

Cholesterol is measured enzymatically in serum or plasma in a series of coupled reactions that hydrolyze cholesteryl esters and oxidize the 3-OH group of cholesterol. One of the reactions by-products H_2O_2 is measured quantitatively in a peroxidase catalyzed reaction that produces color. Absorbance is measured at 500 nm. The color intensity is proportional to cholesterol concentration. The reaction sequence is as follows:

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

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

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

4.7.3.2 Triglycerides

Triglycerides are measured enzymatically in serum or plasma using a series of coupled reactions in which triglycerides are hydrolyzed to produce glycerol. Glycerol is then oxidized using glycerol oxidase, and H_2O_2, one of the reaction products, is measured as described above for cholesterol. Absorbance is measured at 500 nm. The reaction sequence is as follows:

Triglycerides + $3H_2O$ LPL $\longrightarrow$ glycerol + fatty acids

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

Glycerol-3-p + O_2 $\xrightarrow{\text{Glycerophosphate Oxidase}}$ dihydroxyacetone phosphate + H_2O_2

H_2O_2 + 4aminoantipyrine $\xrightarrow{\text{Peroxidase}}$ Quinoneimine + $2H_2O$

Desirable fasting triglyceride levels are considered to be those below 200 mg/dL, and are further categorized as Borderline, 200-400 mg/dL; High, 400-1,000 mg/dL; and Very High (> 1000 mg/dL).

4.7.3.3 High-Density Lipoprotein Cholesterol

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

Principles of the Method

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

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

The reactions are as follows:

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

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

Cholesterol + O_2 $\xrightarrow{\text{PEG cholesterol oxidase}}$ Cholestenone + H_2O_2

In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase Δ4 Cholestenone and hydrogen peroxide.

$2H_2O_2$ + 4-amino-antipyrine +HSDA + H^+ +H_2O peroxidase Purple blue pigment +5 H_2O

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

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

4.7.3.4 LDL-cholesterol

Most of the circulating cholesterol is found in three major lipoprotein fractions: very low density lipoproteins (VLDL), LDL and HDL. LDL-cholesterol is calculated from measured values of total cholesterol, triglycerides and HDL cholesterol according to the Friedewald equation: [LDL C] = [Total Cholesterol] - [HDL] - [TG]/5 Where [TG]/5 are an estimate of VLDL-C and all values are expressed in mg/dL. The equation is derived from another equation, [Total Cholesterol] = [VLDL-C] + [LDL-C] + [HDL-C], but TG are easier to estimate than VLDL and [TG/5] is a good estimate of VLDL, although the Friedewald equation is not valid for calculating LDL if the serum TG is above 400 mg/dL.

4.7.4 Fasting blood glucose (FBG) Determination Principle

Fasting serum glucose levels was determined by enzymatic glucose oxidase method using commercial reagents kit

Principle: In the Trinder reaction the glucose is oxidized to D-gluconate by the glucose oxidase (GOD) with the formation of hydrogen peroxide. A colorless mixture of phenol and 4 aminoantpyrine (4-AA) is then oxidized by hydrogen peroxide in the presence of the enzyme, peroxidase, to form a red Quinoneimine dye product. The concentration of the coloured product, determined from the optical density change at 540 nm in the reaction, is proportional to the concentration of glucose in the original serum sample.

Glucose + O2 +H2O Glucose oxidase H2O2 +Gluconate

2H2O2 + Phenol + 4-Aminoantipyrine Peroxidase 4-(P-benzoquinone-mono-imino) phenazone+ 4H2O

DECLARATION

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

Name: _____

Signature: _____

Name of the institution: _____

Date of submission: _____

This thesis has been submitted for examination with my approval as University advisor

Name and Signature of the first advisor

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Name and Signature of the second advisor _____

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