HEPATIC, RENAL AND CARDIOVASCULAR BIOMARKERS VARIABILITY IN TYPE 2 DIABETES MELLITUS PATIENTS WITH POOR GLYCEMIC CONTROL ATTENDING KAKAMEGA COUNTY GENERAL HOSPITAL, KENYA.

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A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES IN PARTIAL FULFILMENT FOR THE REQUIREMENTS OF THE AWARD OF MASTER'S DEGREE IN MEDICAL LABORATORY SCIENCES (CLINICAL CHEMISTRY) AT MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY

NOVEMBER, 2024

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DEDICATION

I dedicate this thesis to the family of Mr. Tomas Soita Wang’ere.

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ABSTRACT

Type 2 diabetes mellitus (T2DM) is among the largest global health emergencies of the 21st century and one of the leading cause of death in the world. The prevalence of diabetes is high and projected to increase with 10% by 2025. Poor glycemic control continues to be a major concern with majority of the poor glycemic control cases remaining undiagnosed. Microvascular and macrovascular complications due to diabetes are the major causes of poor prognosis among diabetic patients the main ones being hepatic, cardiovascular complications and diabetic nephropathy. A high percentage of T2DM patients remain undiagnosed during the early onset of the disease hence making it difficult to establish the level of organ damage. Upon diagnosis in such cases, hepatic, renal and cardiovascular injury is already evident, and some patients have end-stage organ damage/ failure. Early determination of organ damage plays a critical role in the management of the disease thus preventing related complications and mortality. This study aimed at determining hepatic, renal and cardiovascular decline by evaluating the dynamics and variability of biomarkers in diabetes patients with poor glycemic control. In the study, 103 consenting participants were recruited at the Kakamega county general hospital comprising of (Healthy control, n=27), (T2DM good glycemic control, n=25) and (T2DM poor glycemic control, n=51). This study was an analytical crossectional study and utilized a random sampling technique in participant's recruitment. 6 milliliters of blood sample was collected from the study participants and processed for biomarkers of hepatic, renal and cardiovascular function using colorimetric, spectrophotometric, and florescence immunodetection techniques. Data obtained was cleaned, entered into excel, coded and analyzed using the IBM SPSS 22 software. The normality test was done using the Kolmogorov Smirnov test. Chi-square test was done on categorical variables while Kruskal-Wallis test was done on the continuous variables. A Bonferroni Post-hoc test was done to determine the differences between the groups. The study revealed a significant hepatic biomarker variability in GGT ($P=0.031$), Total bilirubin ($P<0.0001$), Direct bilirubin ($P<0.0001$), albumin ($P=0.001$) and AST/ALT ratio ($P<0.0001$). Renal biomarkers including Urea ($P=0.002$), potassium ($P=0.0012$), sodium ($P<0.0001$) and chloride (0.007) showed a significant variability in poor glycemic control. Additionally, Triglycerides($P<0.0001$), total cholesterol($P=0.046$) and LDH ($P=0.005$) levels were significantly elevated in poor glycemic control. These findings suggest that poor glycemic control causes a significant variability in biomarkers due to the resultant organ damage and these biomarkers can be utilized for determination and early diagnosis of poor glycemic control related organ failure, utilization in disease monitoring and determination of clinical outcomes in T2DM.

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ABBREVIATIONS

ACE: Angiotensin Converting Enzyme

AGE: Advanced Glycation End Products

BMI: Body Mass Index

BUN: Blood Urea Nitrogen

CDC: Center for Disease Control and Prevention

CVD: Cardiovascular Disease

DM: Diabetic Mellitus

DN: Diabetic Nephropathy

GABA: Gamma amino butyric acid

GDF-15: Growth differentiation factor-15

GLUT-4: Glucose Transporter - 4

HbA1c: Glycated Hemoglobin

H-FABR: Heart Type Fatty Acid Binding Receptor

ICAM-1: Intracellular Adhesive Molecules-1

IDF: International Diabetic Federation

ISE: Ion Selective Electrode

MOH: Ministry of Health

NADPH: Reduced Nicotinamide Adenine Dinucleotide phosphate

OGTT: Oral Glucose Tolerance Test

RAAS: Renin Angiotensin Aldosterone System

RNA: Ribonucleic Acid

ROS: Reactive Oxygen Species

T2DM: Type 2 Diabetes Mellitus

VCAM-1: Vascular Cellular Adhesion Molecules-1

VEGF: Vascular Endothelial Growth Factor

WHO: World Health Organization

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OPERATIONALIZATION OF TERMS

Biomarker- this is a measurable indicator of a certain biological process whose levels can be used to diagnose, prognosis, or predict the occurrence of the process in the body

Good glycemic control- this is the state of normal glucose concentration over a period of time which is determined by a HbA1c test value of less than 7%.

Poor glycemic control- Consistently high sugar levels in T2DM patients that are is defined by a HbA1c value of greater than 7.0%.

Variability- Refers to fluctuations, either increase or decrease in the levels of a substance compared it their levels in another group

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Diabetes mellitus is a group of metabolic disorders characterized by a constant hyperglycemic states due to an absolute or relative deficiency of insulin production or action (Alam *et al*., 2014). Type 2 Diabetes mellitus is the most common type of diabetes whose cause is a combination of resistance to insulin action and an inadequate compensatory insulin secretory response (ADA, 2007). Symptoms of marked hyperglycemia include polyuria, polydipsia, weight loss, sometimes polyphagia, and blurred vision (Chatterjee *et al*., 2017). Impairment of growth and susceptibility to certain infections may also accompany chronic hyperglycemia (Banday *et al*., 2020). Acute, life-threatening consequences of uncontrolled diabetes are hyperglycemia with ketoacidosis or the non-ketotic hyperosmolar syndrome (Adeyinka & Kondamudi, 2022). The chronic hyperglycemia of diabetes mellitus is associated with end-organ damage, dysfunction, and failure, including the retina, kidney, nervous system, heart, and blood vessels ("IDF Diabetes Atlas," 2017).

The prevalence and death due to diabetes globally has been increasing over the recent decades. Currently, about 462 million people worldwide have diabetes relating to 6.28% of the world's population (4.4% of those aged 15-49 years, 15% of those aged 50-69, and 22% of those aged 70+). The majority living in third world countries, and 1.5 million diabetes related deaths each year (Khan *et al*., 2020). The worldwide projection of people living with diabetes is 643 million by 2030 and 783 million by 2045 (IDF, 2021). The prevalence of diabetes mellitus in the sub Saharan Africa (SSA) is estimated at 7.1% with projections showing an increase to 10% by 2025 (Zhou *et al*., 2016). As per the World Health Organization (WHO), the prevalence of diabetes in Kenya is at

3.3-5% with over 44% of cases still undiagnosed (Mohamed *al et*., 2018a). Type 2 diabetes accounts for 90-95% of all diabetic cases worldwide and it is more prevalent in adult population (Berezin & Berezin, 2020).

Poor glycemic control is a condition characterized by a constant state of hyperglycemia (FBS, >7.22 mmol/l or HbA1c >7.0%) (Alzahrani *et al*., 2019; Kohnert *et al*., 2015). Regulation and control of normoglycemia remains a challenge in clinical practice since it's a multifactorial issue involving both patient, healthcare personnel and environmental factors (Haghighatpanah *et al*., 2018). Uncontrolled hyperglycemia in T2DM remains a major predisposing factor in development of both microvascular and macrovascular complications (Chawla *et al*., 2016).

Hepatic, Renal and Cardiovascular (CV) function is highly impaired in individuals suffering from T2DM due to various pathophysiological mechanisms (Papatheodorou *et al*., 2018). Cardiovascular events associated with the development of micro and macrovascular dysfunction, acceleration of atherosclerosis, an impaired endogenous repair system, direct cell metabolism impairment, oxidative stress, vascular and systemic inflammation, inadequate immune response, cardiac biochemical stress, fibrosis, necrosis, and apoptosis as well as thrombophilia and aggregation of blood cells (Charlton *et al*., 2021; Fan, 2017; Fowler, 2008).

Renal and cardiovascular damage accounts for approximately 80% of the total mortality cases in T2DM (Papatheodorou *et al*., 2018). While hepatic dysfunction accounting for 12.5% deaths among T2DM patients (Tolman *et a*l., 2007). Poor glycemic control accelerates this damage. A high percentage of T2DM patients remain undiagnosed during the early onset of the disease hence making it difficult to prevent these complications. Upon diagnosis in such cases, organ injury is already evident, and some patients have end-stage organ damage (Pálsson & Patel, 2014). Early

diagnosis of organ damage plays a critical role in the management of the disease thus preventing related complications and mortality (Braunwald, 2019).

Conventional biochemical markers used for renal and cardiovascular diagnosis can also be used to predict renal and cardiovascular function decline in T2DM patients if detected early upon diagnosis (Gowda *et al.*, 2010; Bansal *et al*., 2019). Determining the sensitivity, specificity and predictive value of these biomarkers is essential in the management of T2DM and hence prevents the development and progression to renal and cardiovascular damage. This has a potential of improving the quality of life in diabetic patients thus reducing the health and economic burden associated with T2DM (Berezin & Berezin, 2020).

The conventional biomarkers used for diagnosis of renal injury include creatinine, urea and electrolytes which are routinely done for determination of the renal function at baseline levels. The biomarkers selected for cardiovascular damage and function include Creatine kinase (CK-MB), aspartate transferase(AST), Lactate dehydrogenase 2 (LDH-2), low density lipoproteins (LDL), high density lipoprotein(HDL), triglycerides and total cholesterol (Niizuma *et al*., 2017). Hepatic damage biomarkers include aspartate transferase (AST), alanine transferase(ALT), gamma glutamyl transferase (GGT), Total bilirubin, direct bilirubin, total protein and albumin (Fu *et a*l., 2020). Utilization of these biomarkers in diagnosis of these conditions is usually limited in clinical setting especially in T2DM patients with poor glycemic control (Tummalapalli *et al*., 2020).

The aim of this study was to determine the serum levels of various selected biomarkers for hepatic renal and cardiovascular function, their variability in poorly controlled glycemic state among T2DM patients. This has an overall impact in early organ damage detection, prevention and proper management of T2DM.

1.2 Problem statement

T2DM has been on a constant rise worldwide, with increasing annual mortality rates. This has been attributed to its effects on organs through various pathophysiological processes. Persistent hyperglycemia predominantly affects the hepatic, renal and cardiovascular systems. Consequently, failure of these systems promotes the progression of diabetes mellitus with a subsequent increase in mortality. To date, management of T2DM majorly focuses on rectification of hyperglycemia, however, there has been an increased report of poor glycemic control in T2DM thus posing a great risk. In Kenya, only 36.6% of the patients who receive diabetic care achieve optimal glycemic control (Otieno *et al*., 2021). Additionally, organ damage in diabetes usually goes undetected since it is difficult to detect clinically in early stages without proper laboratory biomarker determination. There is also little focus on the detection, management, and prevention of organ failure especially in absence of clinical features suggesting the damage. Early diagnosis of DM and progressive monitoring of organ damage bears the potential to improve management of T2DM complications, therefore, reducing mortality rates and management burden due to organ failure. Several biomarkers of renal and cardiovascular damage have been established in the literature and practice. The use of these biomarkers is mainly reserved for determining the end-stage organ damage. Nonetheless, there is a paucity of information on the use of these biomarkers in early determination of disease of progression and predicting the future organ damage especially among individuals with uncontrolled glycaemia. This has made it difficult to manage the consequences of hyperglycemia on these organs thus increasing the health management and economic burden. In literature, more studies focus on understanding the factors that are associated with poor glycemic control. However, early biomarker variability determination has remained a challenge in practice

especially in third world countries. Therefore, this study aimed at determining the various hepatic, renal and cardiovascular biomarker variability among the T2DM patients with poor glycemic control with the main aim of increasing early diagnosis, determining disease progression and outcome.

1.3 Objectives

1.3.1 General objective

To determine hepatic, renal and cardiovascular biomarkers variability among type 2 diabetes mellitus patients with poor glycemic control attending Kakamega county general hospital.

1.3.2 Specific objectives

1. To determine the hepatic biomarkers variability among type 2 diabetes mellitus patients with poor glycemic control attending Kakamega county general hospital.
2. To determine the renal biomarkers variability among type 2 diabetes mellitus patients with poor glycemic control attending Kakamega county general hospital.
3. To determine the cardiovascular biomarkers variability among type 2 diabetes mellitus patients with poor glycemic control attending Kakamega county general hospital.

1.4 Null hypotheses

1. Poor glycemic control has no effect on the hepatic biomarker variability among patients with type 2 diabetes mellitus.
2. Poor glycemic control has no effect on the renal biomarker variability among patients with type 2 diabetes mellitus.
3. Poor glycemic control has no effect on the cardiovascular biomarker variability among patients with type 2 diabetes mellitus.

1.5 Justification

Diabetes mellitus has reached epidemic proportions and affects more than 420 million individuals worldwide. Global estimates predict an increase of 50% by the year 2035, with the greatest increase in the developing countries of Africa, Asia, and South America. T2DM accounts for above 85-90% of all diabetes cases in the country and is a major cause of diabetes-related complications. Hepatic, renal and cardiovascular systems are the major sites affected by persistent hyperglycemia occurring in 50-60% of these patients and also accounting for a major cause of death in these patients. Poor glycemic control remains a challenge both worldwide and nationally with over 64% of diabetic patients being unable to regulate their blood sugar levels. In T2DM, the majority of patients have organ injury at the time of diagnosis as T2DM can go unrecognized for many years. This necessitates the development of early risk detection measures thus enabling proper management of these complications. Thus understanding and analyzing routine biochemical markers bears the potential of necessitating early diagnosis, promote diseases progression monitoring and thus leading to early prevention and proper management of T2DM patients. Studies have indicated that factors such as educational awareness, economic challenges and reduced healthcare seeking behaviors as witnessed in rural settings play a key role in poor glycemic control. Kakamega is one of the most populated rural county in Kenya and recent data shows that the odds of good glycemic control among patients in rural areas are 47.2% lower as compared to urban areas. This study has a potential of improving healthcare management through understanding the biomarkers for early detection of poor glycemic control and associated complications thus improving T2DM related outcomes and the lowering the healthcare economic burden.

1.6 Study Significance

This study provides an additional information on the burden of poor glycemic control among T2DM patients. It also sheds light on extend of organ damage especially in unregulated hyperglycemia which in turn stresses on the importance of adherence to treatment, continuous glycemic monitoring and regular visiting of diabetes clinics for T2DM patients. The findings from the study reveals that biomarkers such as urea, chloride, bilirubin direct, bilirubin total, total cholesterol and triglycerides are potential biomarkers of organ damage and also indicators of poor glycemic control in T2DM patients. The study forms a basis for formulation and improvement of existing diabetic care guidelines, education materials and also basis for reference in diabetic care counseling. Early determination of potential organ injury and progression through these biomarkers has an overall impact of reducing healthcare-related burden through early interventions and improving the quality of life in diabetic patients.

1.7 Limitations of the study

The study relied on self-reported period since initial diagnosis which may not be sufficient and could bring bias.

The study only utilized HbA1c as the only index to classify glycemic control. Despite it being the gold standard, other indices such as glycated albumin, fructosamin and 1,5-anhydroglucitol used in combination could have been more appropriate.

The intervening variables such as Age and Gender were not matched in the study which made it difficult to control their direct effect on the various biomarkers that were measured

1.8 Delimitations

1. Patients initial records were also checked to determine the period of stay with diabetes mellitus since initial diagnosis.
2. HbA1c remains to be the gold standard and most widely used to classify glycemic control thus was appropriately utilized.

1.9 Conceptual framework

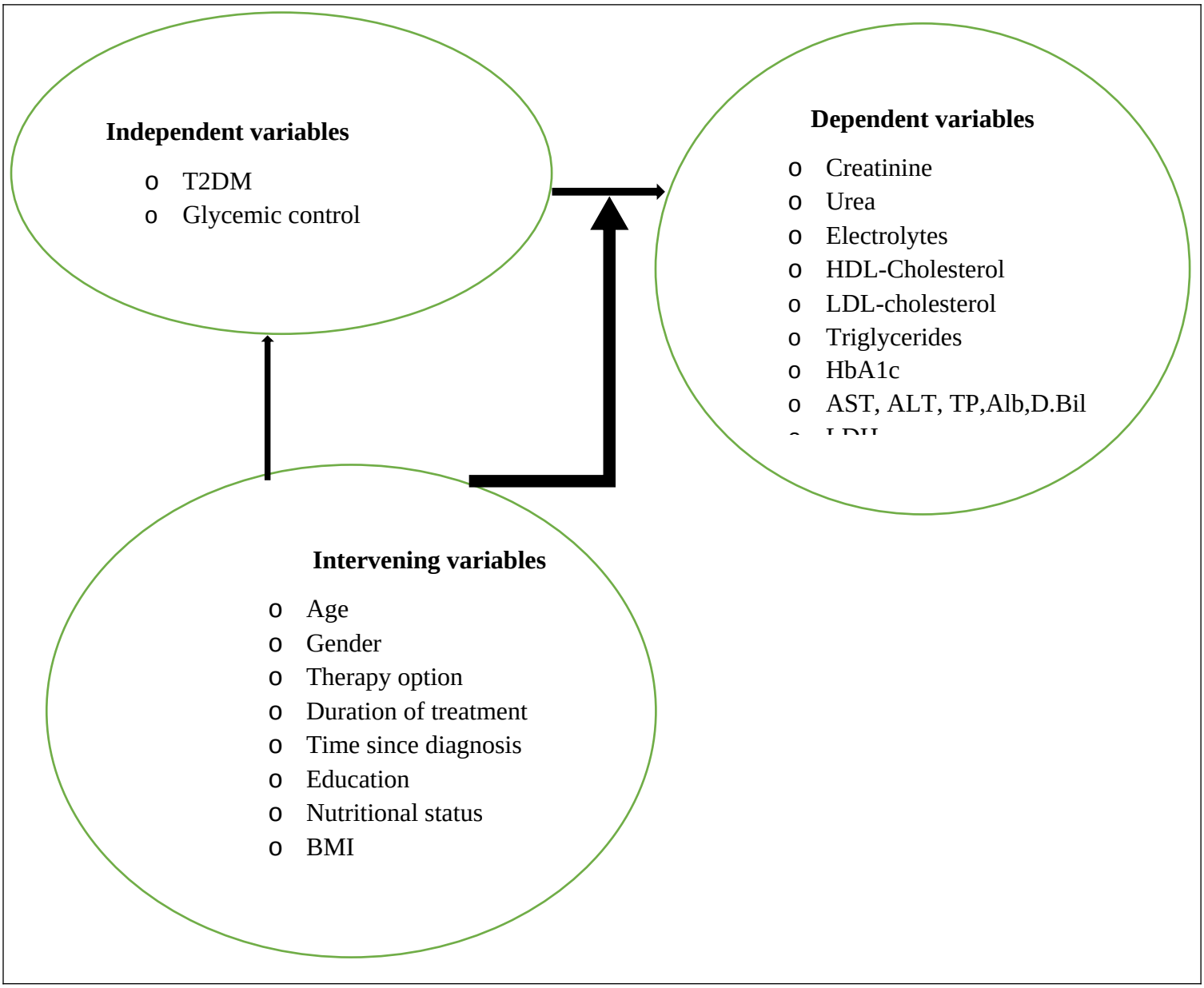

Figure 1.1. A conceptual framework showing the relationship between study variables.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Occurrence and epidemiology

T2DM is now classified as an epidemic, which represents a most significant public health concern globally (Charlton et al., 2021). Currently, 537 million individuals (20-79 years old), or 1 in 10 people worldwide, have diabetes. It is expected that this figure would increase to 643 million by 2030 and 783 million by 2045. Three to four out of every ten diabetic adults reside in low- or middle-income nations. With 15.9 million cases, Africa has a 3.1% prevalence. The majority of undiagnosed cases are concentrated in Africa, and by 2045, global forecasts show an increase of almost 156%. Over the past few years, diabetes mortality rates have significantly increased. In 2021, over 6.3 million deaths were expected, or one patient every three seconds (IDF, 2022).

As per the world health organization(WHO), the prevalence of diabetes in Kenya is at 3.3-5% and is expected to rise to 45% in 2025 (Mohamed et al., 2018). Due to unawareness among Kenyans, two-thirds of diabetic patients are undiagnosed thus making the possibility of eradicating diabetes a more difficult task. Kenya has been ranked by IDF as the 31st African country in terms of diabetes with a prevalence of about 560 diabetic cases per 10,000 population (Katambo, 2021).

Approximately 90% to 95% of diabetic cases are T2DM, low and middle income countries being the most affected. It is a common problem globally that has evolved in association with rapid cultural, economic and social changes, aging populations, increasing and unplanned urbanization, poor dietary intake such us high sugar beverages and processed foods, obesity, physical inactivity, uncontrolled lifestyle and behavioral patterns, fetal malnutrition, and increasing fetal exposure to hyperglycemia during pregnancy. T2DM is most common in adults, but an increasing number of children and adolescents are also affected (Chatterjee *et al*., 2017).

2.2 Risk factors, signs, and symptoms for T2DM

A genetic and metabolic factor interplay determines the risk of T2DM and varies among various populations (Fan, 2017). However, the main risk factors that cut across include the following; positive family history of diabetes, age of greater than 35 years, overweight with a body mass index(BMI) of < 18.5 ("underweight"), 18.5 < 24.9 ("normal weight"), 25 < 29.9 ("overweight"),> 30 (obese), enlarged waist or upper body adiposity (>90cm for men and >80cm for women), hypertension, recent uncontrolled weight gain, sedentary lifestyle, increased physical inactivity, and history of gestational diabetes (Ramachandran, 2014; Stommel & Schoenborn, 2009).

The warning signs of diabetes mellitus are in most cases disregarded due to the chronic nature of the disease and thus remain undiagnosed in the early onset of the disease. This leads to delayed treatment and management thus leading to the development of Diabetes related complications. Reactive hypoglycemia, itching, dry mouth, burning pain or numbness in the feet, frequent fatigue, irritability, repeated infections, particularly in the genital area, urinary tract, skin, and oral cavity, delayed wound healing, and acanthoses nigricans—velvety dark patches on the neck, armpit, and groin that are a sign of insulin resistance—are some of these symptoms (Kerner & Brückel, 2014).

2.3 Diagnosis of diabetes mellitus

WHO recommends diagnostic tests for diabetes mellitus which include the measurement of fasting glucose levels(FBS), 2-hour oral glucose tolerance test(OGTT), HbA1c test and random blood glucose (RBS)in suspected signs and symptoms of diabetes. Individuals with a fasting plasma glucose values of ≥ 7.0 mmol/L (126 mg/dl), 2-h post-load plasma glucose ≥ 11.1 mmol/L (200 mg/dl), HbA1c ≥ 6.5% (48 mmol/mol); or an RBS of ≥ 11.1 mmol/L (200 mg/ dl) with diabetes symptoms are considered to have diabetes. Elevation in asymptomatic prompts repeat of the testing for confirmation (WHO, 2019).

Over the years, RBS, FBS and OGTT have solely been used for the initial diagnosis of diabetes. However, the introduction of HbA1c by the recent WHO guidelines have proven to be more effective in both diagnostic and monitoring of diabetes (Kerner & Brückel, 2014). In diabetes diagnosis using HbA1c test, a specificity of ≥6.5% justifies diabetes diagnosis while a sensitivity of < 5.7% rules out diabetes. HbA1c levels between 5.7 to 6.4% prompts use of traditional criteria for prediabetes and diabetes diagnosis (Kerner & Brückel, 2014).

2.4 Pathophysiology of T2DM

Glucose regulation is done by the insulin hormone through a balanced interplay between its action and secretion (Banday *et al*., 2020). Insulin related anomalies include β-cell dysfunction and insulin resistance. Insulin resistance results from cellular pathways disruptions, leading to a decreased response or sensitivity of cells in the peripheral tissues towards insulin particularly in muscle, liver, and adipose tissue (Kahn *et al*., 2014).

β-cell hyperfunction occurs in early stages triggered by reduced insulin insensitivity achieving a compensatory high insulin secretion to maintain normal glucose levels (Banday *et al*., 2020). The higher levels of circulating (Hyperinsulinemia), thus prevent hyperglycemia. However, gradually, the increased insulin secretion by β-cells is not able to compensate sufficiently for the decrease in insulin insensitivity. Moreover, the β-cell function begins to decline and β-cell dysfunction eventually leads to insulin deficiency. As a result, normoglycemia can no longer be maintained and hyperglycemia develops.

B-cell dysfunction is a critical component in T2DM pathophysiology, reduced insulin secretion is due to a reduction in the number of β-cells or the β-cells inability to secrete enough insulin in quantities enough to cause normoglycemia (Lim, 2014). The reduced number of β-cells has a multifactorial basis, which includes glucolipotoxicity (Poitout *et al*., 2010) and amyloid deposition

(Jurgens *et al*., 2011) that results in β-cell apoptosis through oxidative and endoplasmic reticulum stress. These cells once lost are not renewed due to pancreatic incapability to do so in individuals beyond 30 years of age (Kahn *et al*., 2014).

While less well studied, dysregulated glucagon release by the α-cell leads to high fasting glucagon concentrations and a failure to revert the glucagon release process after a meal leads to hyperglycemia (Dunning & Gerich, 2007). Whether this represents a primary change in the α-cell or is secondary to an abnormality in β-cell function is not yet resolved. It is known that islet blood flows from the β-cell to the α-cell and then to the somatostatin-producing δ-cell, and that the high concentrations of insulin bathing the α-cell are capable of suppressing glucagon release (Gromada *et al*., 2007). Zinc, γ-amino-butyric acid (GABA) and glutamate are β-cell products involved in regulation of glucagon release (Gromada *et al*., 2007). Treatment alternative of T2DM may involve targeting glucagon release by either diminishing its release or impair the action to elevate glucose levels (Dunning & Gerich, 2007).

Insulin peripheral resistance is a state experienced when insulin effects are less than what's expected for glucose uptake/disposal in skeletal muscle cells and suppression of endogenous glucose production primarily in the liver. In the fasting state, however, muscle accounts for only a small proportion of glucose disposal (less than 20%) whereas endogenous glucose production is responsible for all the glucose entering the plasma. Endogenous glucose production is accelerated in patients with type 2 diabetes or impaired fasting glucose. Because this increase occurs in the presence of hyperinsulinemia, at least in the early and intermediate disease stages, hepatic insulin resistance is the driving force of hyperglycemia of T2DM (Czech, 2017).

2.5 Poor glycemic control

Poor glycemic control is a state of unregulated hyperglycemia with or without treatment. This can be determined through a HbA1c test with persistent results of >7.0% and persistent high random blood sugar of >8mmols/l (Alzahrani *et al*., 2019; Kohnert *et al*., 2015) Disease progression, complications and even death in T2DM is directly linked to glycemic control thus making it a major component of diabetic care (Mamo *et al*., 2019). The therapeutic aim of T2DM treatment is rectification of hyperglycemia thus persistence creates a major management concern. Development of poor glycemic control is multifactorial and remain complex to understand. These factors include poor adherence to glycemic therapy, unregulated diet, physical inactivity, young age, period since diagnosis/ time lived with DM, treatment options, monotherapy or combination therapy, smoking, hypertension and level of education all are associated with poor glycemic control (Haghighatpanah *et al*., 2018; Mamo *et al*., 2019; Wagai & Romshoo, 2020).

2.6 Treatment of T2DM

T2DM treatment guidelines emphasize on early, effective and sustained control of sugar levels to delay the onset, reduce the disease severity and delay onset of diabetic related complications. Most of the guidelines favor an individual targeted approach to treatment underpinned throughout by lifestyle measures, notably diet, exercise, body weight control, and healthy living advice. The blood glucose management protocol is recommended for patients with established or newly diagnosed T2DM. WHO recommendations involve both non-pharmacological and pharmacological management (Sarker *et al*., 2020).

2.6.1 Non pharmacological management

In order to control the risks and lessen the severity of the disease in those who are already afflicted, non-pharmacological management of diabetes is crucial. Since it incorporates lifestyle changes

like eating a balanced diet and maintaining a normal body weight through regular exercise like weightlifting, jogging, or walking, it is the most advised kind of management. Cutting back on high-calorie foods significantly lowers weight, which lowers the BMI. Additionally, patients are counseled to cut back on or abstain from alcohol and tobacco usage (Raveendran *et al*., 2018).

2.6.2 Pharmacological management

The WHO recommends changing one's lifestyle as the first line of treatment for type 2 diabetes. When diet alone is unable to control type 2 diabetes in adults without renal insufficiency, hepatic disease, or hypoxia, metformin is typically used as a first-line oral antidiabetic medication. Patients for whom metformin is contraindicated or does not improve glycemic control may be prescribed a sulphonylurea. Referrals to the next level of care (such as combination therapy) should be made for patients whose fasting plasma glucose levels remain uncontrolled even after taking the maximum amounts of metformin or sulphonylureas (Harding *et al*., 2019)

A number of blood sugar-lowering drug classes have been approved for use in combinational therapy and as oral and subcutaneous injections. Their uses vary according on the patient (Bailey & Day, 2018).

Metformin is an example of biguanides that is the main first-line oral drug of choice in the management of T2DM across all age groups (Bailey & Day, 2018). Through intricate effects on the mitochondrial enzymes, metformin causes the liver's adenosine monophosphate-activated protein kinase to become active, which results in hepatic uptake of glucose and inhibits gluconeogenesis. Metformin has few adverse effects, a low risk of hypoglycemia, and little probability of weight gain. It is also very well tolerated. By lowering hepatic glucose synthesis (gluconeogenesis) and making peripheral tissues more sensitive to insulin, metformin has been demonstrated to slow the advancement of type 2 diabetes, lower the risk of complications, and lower patient mortality rates

(Viollet *et al*., 2011). Additionally, it increases tyrosine kinase activity and activates insulin receptor expression, which enhances insulin sensitivity. According to recent data, metformin also protects CVDs by lowering plasma lipid levels via the peroxisome proliferator-activated receptor (PPAR)-α pathway. The incretin-like effects of glucagon-like peptide-1 (GLP-1) may be the cause of decreased food intake. Therefore, in overweight and obese people at risk for diabetes, metformin may provide modest weight loss (Viollet *et al*., 2011).

By inhibiting the KATP channels, sulfonylureas increase the pancreatic production of insulin, lowering blood glucose levels. They also restrict the liver's gluconeogenesis. Sulfonylureas impair the liver's ability to eliminate insulin and break down fats into fatty acids. Currently, sulfonylureas are recommended as supplemental or second-line treatments for type 2 diabetes. Glipizide, glimepiride, and glyburide are examples of second-generation medications, while chlorpropamide, tolazamide, and tolbutamide are examples of first-generation agents. Compared to second-generation sulfonylureas, first-generation sulfonylureas are known to have slower onset of action, longer half-lives, and a higher risk of hypoglycemia (Ismail-Beigi, 2012). Second-generation sulfonylureas are currently recommended and preferred over first-generation drugs in clinical practice because to their shown potency (given to patients at lower doses and low frequency), with glimepiride having the safest profile. The most common adverse effect of all sulfonylureas is hypoglycemia, while other common side effects include headache, nausea, dizziness, hypersensitivity reactions, and weight gain. (Chaudhury *et al*., 2017).

Incretin Mimetics; The differential in insulin secretory response between oral and intravenous glucose loads is known as the "incretin effect" or "incretin mimetics." After consuming glucose orally, 50–70% of total insulin release is caused by the incretin effect. Glucagon-like peptide (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP, or incretin), two naturally

occurring incretin hormones that are crucial for maintaining glycemic control, have a brief half-life because dipeptidyl peptidase 4 (DPP-4) inhibitors hydrolyze them in less than one and a half minutes. The incretin effect is diminished or nonexistent in T2DM patients. Specifically, people with type 2 diabetes lose GIP's insulinotropic effect. Incretins lead to weight loss by slowing down stomach emptying. These drugs are becoming more and more popular in the treatment of diabetes because of their effect on weight loss. Incretin system targeting has emerged as a key therapeutic strategy for the treatment of type 2 diabetes. GLP-1 receptor agonists and DPP-4 inhibitors are two examples of these medication classes. According to clinical research, these treatments help patients with type 2 diabetes maintain their blood sugar levels while lowering their body weight (particularly, GLP-1 receptor agonists) and systolic blood pressure (Nisa M *et al.,* 2016).

Thiazolidinedione (TZDs) improve insulin action just like biguanides. Two examples of thiazolidineodione are pioglitazone and rosiglitazone. As PPAR (peroxisome proliferator-activated receptor) agonists, TZDs promote greater glucose absorption in a variety of organs, such as the liver, muscle, and adipose tissue. Insulin resistance and β-cell exhaustion are improved via mechanisms of action that include lowering the buildup of free fatty acids, lowering inflammatory cytokines, increasing adiponectin levels, and maintaining β-cell integrity and function. But there are serious worries that the risks will outweigh the advantages. In particular, heart failure is brought on by combination insulin-TZD treatment. Therefore, it is not recommended to use TZDs as first-line or even step-up therapy (Chaudhury *et al*., 2017).

A second oral medication, a GLP-1 receptor agonist, or basal insulin, such as Mixtard, may be added to the regimen if non-insulin monotherapy, such as metformin at the maximum permitted dose, is unable to reach or sustain the HbA1c target after three months. Patients with newly diagnosed type 2 diabetes who exhibit unmistakable symptoms (such as catabolic features like

weight loss, ketosis, or hyperglycemia features like polyuria/polydipsia) and/or significantly elevated blood glucose levels [≥300–350 mg/dL (16.7–19.4 mmol/L)] should be started on insulin therapy, either with or without additional agents. or HbA1c [≥10–12%] (Bailey & Day, 2018).

Meglitinide interacts to the sulfonylurea receptor in the pancreatic β-cells via the same mechanism as sulfonylureas. But because meglitinide binds to the receptor less strongly than sulfonylurea, it is regarded as a short-acting insulin secretagogues, which allows for more administration flexibility (Chaudhury *et al*., 2017).

Glucosuric drugs known as SGLT2 inhibitors reduce blood sugar levels by preventing the proximal nephron tubules from reabsorbing glucose. These medications work better in later or more advanced stages of type 2 diabetes after the pancreatic β-cell reserves have been permanently depleted. The mechanism is insulin-independent. Dapagliflozin and canagliflozin are two examples of such medications, and using them is linked to lowering blood pressure and losing weight (Riser Taylor & Harris, 2013).

2.7 Cardio-renal complications in T2DM

Among type 2 diabetes mellitus patients' primary causes of death are cardiovascular and renal problems (Gilstrap & Wang, 2012; Lim, 2014). Microvascular and macrovascular issues are linked to cardiovascular and renal problems in type 2 diabetes, respectively (Fowler, 2008). Heart metabolic disorders, abnormalities in subcellular signaling, autonomic dysfunctions, activation of the renin-angiotensin-aldosterone system (RAAS), inflammation, oxidative stress, and maladaptive immunological response are some of the pathogenic pathways that lead to these issues(Charlton *et al*., 2021).

2.7.1 Cardiovascular pathophysiology in T2DM

Cardiac complications such as heart failure have a prevalence of 22% among T2DM patients (Berezin *et al*., 2022). Regardless of age, blood pressure, BMI, or the presence of coronary artery disease (CAD), a 1% increase in HbA1c is linked to an 8% increase in the risk of heart failure. Insulin resistance and hyperglycemia are two characteristics specific to type 2 diabetes that influence heart failure. A 16% lower risk of heart failure and worsened outcomes is linked to a 1% decrease in HbA1c (Braunwald, 2019). The following are the primary pathophysiological pathways of cardiac diseases in diabetes: inflammation, oxidative stress, autonomic dysfunction, subcellular signaling abnormalities, cardiac metabolic changes, and maladaptive immunological response. A wide spectrum of damage to the heart and vasculature, such as atherosclerosis, coronary heart disease, myocardial infarction, and cardiomyopathy, are included in cardiovascular disease (CVD), a complex illness (Charlton *et al*., 2021). Myocardial fibrosis, dysfunctional remodeling, and related diastolic dysfunction are the early signs of diabetic cardiomyopathy. Systolic dysfunction follows, and ultimately, clinical heart failure (Berezin *et al.*, 2022).

2.7.1.1 Subcellular signaling abnormalities

Through regulation of protein synthesis, substrate use, and cell survival, cardiac insulin signaling mediates cellular homeostasis. Similar to skeletal muscle, liver, and adipose tissue, GLUT4 mediates glucose transport in cardiac tissue. Insulin stimulates GLUT4 translocation to the cell membrane and subsequent glucose absorption by binding to the insulin receptor and activating the insulin signaling/docking molecule insulin receptor substrate (IRS)-1/2 and downstream PI3K/protein kinase B (Akt) (Jia *et al*., 2016). Additionally, endothelial nitric oxide synthase (eNOS) activation and bioavailable nitric oxide (NO), which are essential for good coronary microvascular flow and myocardial function, are promoted by appropriate coronary artery and

myocardial insulin metabolic signaling (Jia *et al*., 2015, 2016) Cardiac insulin receptor deletion causes mitochondrial dysfunction, increases the generation of reactive oxygen species (ROS), and lowers the absorption of glucose by the heart (Bugger *et al*., 2012). Double IRS-1/2 deletion increases fibrosis and heart failure, decreases cardiomyocyte ATP content, and affects cardiac metabolism and function (Bugger *et al*., 2012). Ventricular muscle biopsies from patients with type 2 diabetes mellitus have also shown decreased PI3K/Akt signaling as well as decreased GLUT4 expression and translocation (Bertoni *et al.*, 2006).

Mitsugumin 53, an E3 ubiquitin ligase, may have a significant detrimental effect on insulin signaling maintenance. Increased proteasomal degradation of the insulin receptor and IRS-1 was linked to elevated cardiac mitsugumin 53 protein levels in the type 2 diabetes mouse model (Jia *et al.*, 2018). Additionally, cardiomyocyte-specific overexpression of mitsugumin 53 exacerbated cardiac fibrosis and suppressed insulin signaling, indicating that cardiac mitsugumin 53 downregulation may be a viable therapeutic approach to prevent diabetic cardiomyopathy and the development of clinically evident heart failure. Through increased activation of the mammalian target of rapamycin (mTOR)/S6 kinase 1 signaling pathway, which raises serine phosphorylation and decreases tyrosine phosphorylation of IRS-1/2, and hinders PI3K engagement, Akt/eNOS activation, and NO production, risk factors like obesity and inappropriate RAAS activation can impair cardiac insulin metabolic signaling. Reduced NO production affects insulin-mediated capillary recruitment and coronary artery relaxation, both of which are critical for the supply of insulin and glucose required for healthy myocardial energetics (Jia *et al*., 2015, 2016). Heart fibrosis and stiffness are promoted by impaired NO production since it also increases the activation of collagen cross-linking enzymes such transglutaminase (Bertoni *et al*., 2006). Proinflammatory cytokines, like tumor necrosis factor α, have been shown to cause cardiac insulin resistance by

activating cJun N terminal kinase (JNK), which phosphorylates IRS-1, and nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) (Jia *et al*., 2018).

2.7.1.2 Oxidative stress

An imbalance between the body's generation of reactive oxygen species (ROS) and antioxidant activity results in oxidative stress, which causes ROS to build up and cause direct harm to cells and tissues (Pizzino *et al*., 2017). Superoxide (·O2), peroxyl (ROO·), hydroxyl (·OH), and hydrogen peroxide (H2O2) are examples of ROS, which are tiny molecules that are energetic and reactive that are produced from oxygen (Jha CJ *et al.*, 2016). Furthermore, pro-inflammatory cytokine activation brought on by oxidative stress leads to inflammation, which in turn increases ROS generation and damages cells and tissues (Oguntibeju 2019). The polyol, hexosamine, and protein kinase C (PKC) pathways, the NADPH oxidase family, and/or the buildup of advanced glycation end products (AGEs) are the processes that increase ROS production at the cellular level. The aforementioned pathways are activated by chronic hyperglycemia, which raises ROS generation and aggravates cardiovascular and renal damage (Charlton *et al*., 2021).

2.7.1.3 Macrovascular complications

The central pathologic mechanism is atherosclerosis which leads to the narrowing of arterial walls throughout the body (Fowler, 2008). This process is started by endothelial dysfunction, which permits lipids to enter the vessel wall, where they undergo oxidation and release cytokines that promote inflammation. Leukocytes and monocytes are drawn in as a result, and these cells develop into macrophages and foam cells, which further produce cytokines and aid in the development of atherosclerotic plaque and an inflammatory cycle. As smooth muscle cells migrate, proliferate, and collagen builds up over time, this plaque gradually thickens and eventually calcifies to create an atherosclerotic, lipid-rich lesion with a fibrous capsule. Acute arterial infarction and thrombus

formation are triggered by the disruption and rupture of this lesion, even if plaque growth itself may block the artery lumen and hinder vascular flow (Charlton *et al*., 2021).

Type 2 diabetes is strongly associated with enhanced platelet adhesion and hypercoagulability in addition to atheroma development. Platelet aggregation may be encouraged by decreased nitric oxide production, elevated free radical creation in platelets, and changed calcium regulation. Patients with diabetes may also have impaired fibrinolysis due to elevated levels of plasminogen activator inhibitor type 1. In type 2 diabetes, the risk of arterial occlusion and cardiovascular events is probably further enhanced by the combination of increased coagulability and impaired fibrinolysis (Beckman *et al*., 2002).

2.7.1.4 Inflammation

By producing cytokines and expressing adhesion molecules like VCAM-1 and ICAM-1 that improve cell retention within the lesion, vascular smooth muscle cells (VSMCs) contribute to an inflammatory cascade (Zakai *et al*., 2007). Furthermore, ROS and AGE enhance each other's effects in both directions, and both substances increase LDL oxidation, which is known to increase the production of MCP-1, ICAM-1, and VCAM-1 (Wu & Parhofer, 2014). Furthermore, oxidative stress linked to hyperglycemia promotes the activation of pro-inflammatory signaling pathways, such as NFκB, mitogen-activated protein kinase (MAPK), or PKC, which further accelerates the development of atherosclerosis (Viigimaa *et al*., 2020). Additionally, ROS mediates a number of signaling pathways that support the development of lesions and, eventually, the rupture of plaque. For example, AGE can accelerate the development of atherosclerosis by causing plaque to calcify. When it interacts with the receptor for AGE (RAGE), adhesion molecules and cytokines are produced, which maintain an inflammatory environment and accelerate the development and progression of atherosclerotic lesions (Jandeleit-Dahm *et al.*, 2008). Furthermore, diabetes-related

oxidative stress raises the risk of thrombosis by causing platelets to become hyper-reactive. The common combination of oxidative stress, hyperglycemia, and increased vascular shear stress in T2DM patients exacerbates this platelet hyper-reactivity (Gaiz *et al*., 2017).

2.7.2 Diabetic nephropathy pathophysiology

The primary cause of renal failure is diabetic nephropathy (DN), a microvascular consequence of diabetes. In the context of diabetes, it is characterized by proteinuria over 500 mg in a 24-hour period; however, smaller levels of proteinuria or microalbuminuria occur before this (Fowler, 2008). It has been reported that 7% of patients with T2DM already have microalbuminuria at the time they are diagnosed with diabetes (Chaturvedi N *et al.*, 2001). A number of structural and functional alterations are hallmarks of diabetic nephropathy (DN). The hallmarks of glomeruli include mesangial enlargement, basement membrane thickening, and nodular glomerulosclerosis (Kimmelstiel–Wilson nodules). Early DN is characterized by tubular hypertrophy, but interstitial fibrosis with tubular atrophy and arteriolar hyalinosis gradually emerge. In more severe cases, T lymphocytes and macrophages invade. Reduced endothelial cell fenestration and podocyte loss are observed ultrastructurally. When nephropathy progresses, there is a decrease in glomerula filtration rate (GFR), an increase in proteinuria, and early glomerular hyperfiltration and increased albumin excretion (Weil *et al*., 2012).

2.7.2.1 Hemodynamic mechanisms

When afferent and efferent arteriolar resistance are out of balance, hemodynamic mechanisms cause hyperfiltration and elevated glomerular hydrostatic pressure, which in turn causes aberrant renal function. Additionally, the Renin-angiotensin aldosterone system (RAAS) is activated, raising angiotensin II levels, which causes efferent arteriolar vasoconstriction and the generation of proinflammatory and profibrotic chemicals via a variety of pathways. In both people and diabetic

mice, increased ACE activity is linked to increased albuminuria and nephropathy (Wang *et al*., 2018). Vasoconstriction is additionally influenced by elevated levels of urotensin II and endothelin-1. In DN, several forms of nitric oxide and nitric oxide synthase dysregulation have been reported. Nitric oxide is produced from L-arginine by endothelial nitric oxide synthase and facilitates endothelium-dependent vasodilatation (Charlton *et al*., 2021).

2.7.2.2 Metabolic mechanisms

Reactive oxygen species (ROS) and oxidative stress can harm proteins and DNA or act as signaling amplifiers to trigger cellular stress pathways like nuclear factor kappa B (NF-κB), mitogen-activated protein kinases (MAPK), and protein kinase C (PKC) (Charlton *et al.*, 2021). By raising the NADH/NAAD+ ratio, activation of the polyol pathway—in which excess glucose is converted to sorbitol by aldose reductase and then converted to fructose by sorbitol dehydrogenase—contributes to oxidative stress (Fowler, 2008).

In addition to the previously mentioned elements, proinflammatory cytokines are produced and innate immune cells are recruited and activated. Early diabetic glomeruli are characterized by a high concentration of macrophages and T lymphocytes, with an interstitial infiltration developing later. The degree of glomerulosclerosis in people is correlated with the formation of kidney macrophages. Interstitial fibrosis, proteinuria, and a decrease in GFR were all substantially linked with the accumulation of interstitial macrophages (Lim, 2014). Renal cells are also thought to be injured by glycoproteins. High glucose concentrations can promote the non-enzymatic formation of advanced glycosylated end products(AGEs) which are associated with the formation of microaneurysms and pericyte loss (Fowler, 2008). There is also evidence of activation of growth factors such as VEGF, GH, and TGF-β and its downstream cytokine, CTGF which has been shown to pray a role in the progression of diabetic nephropathy (Duran-Salgado & Rubio-Guerra, 2014).

CHAPTER THREE
3.0 MATERIALS AND METHODS

3.1 Study area

The study was conducted at the Kakamega County General Hospital. The facility is located in the Kakamega County of former Western province. The county covers an area of approximately 3,033.80km^2. Kakamega County has a total population of 1.8 million people according to the data from Kenya National Bureau of Statistics census data in 2019. The population density is 618.4/Km^2 with 1.6 million living in the rural area while 185,340 staying in the urban environment.

The facility serves as a major referral center for various other health facilities both from within the county and from the neighboring counties like Busia, Vihiga, Bungoma and Siaya. The study participants were recruited from the outpatient department and diabetic clinic. The samples collected thereafter were processed and stored at the Kakamega County General Hospital laboratory.

3.2 Study design

The study employed an analytical cross-sectional design where T2DM participants were categorized into two groups (Good glycemic control group and poor glycemic control group) based on the HbA1c levels that was measured during recruitment. A healthy control group was also recruited and subjected to the same conditions and measurements as the T2DM group. Data was collected from participants in each of these groups at a single point in time.

3.3 Study population

The study was conducted among patients diagnosed with Type 2 diabetes mellitus (T2DM) attending the Kakamega county general hospital outpatient and diabetic clinic. Participants recruited were only who met the inclusion criteria.

3.3.1 Inclusion criteria

i. Consenting adults

ii. Type 2 Diabetes mellitus patients, (both newly diagnosed and old diabetic cases).

3.3.2 Exclusion criteria

i. Renal and cardiovascular pathological conditions due to a different etiology.

ii. Use of other non-diabetic-related medications through checking of the medical records.

iii. Other types of diabetes

iv. Other chronic illnesses such as HIV/AIDS and cancer through checking of medical records and testing of HIV/AIDS

3.4 Study variables

3.4.1 Dependent variables

The variables include levels of creatinine, urea, glomerular filtration rate(GFR), lactate dehydrogenase (LDH), creatinine kinase MB (CK-MB), random blood sugar, HbA1c, high-density lipoprotein, low-density lipoprotein(LDL), triglycerides(TGs), total cholesterol(TC), aspartate aminotransferase, serum albumin, total proteins (TP), calcium levels, alanine aminotransferase, gamma glutamyl transferase, sodium, potassium, chloride, total bilirubin and direct bilirubin.

3.4.2 Independent variables

The independent variable in this study is type 2 diabetes mellitus, and glycemic control.

3.4.3 Intervening variables

The intervening variables comprise of age, sex gender, type of diabetic medication being used by the patient, duration of specific treatment use, duration of stay with the disease since diagnosis, educational background, and nutritional characteristics such as BMI.

3.5 Sampling design

The random sampling design was used to select the diabetic patients for participation in the study.

3.6 Sample size determination

The sample size was determined using the Cochran formula where 103 participants were recruited in the study. Calculation for determination is as shown below.

Where: -

Z = is the Z-score of the normal distribution under a 5% error estimate (1.96)

P = prevalence of T2DM in SSA is 5.5% (Pastakia *et al*., 2018)

q =1-p

d= standard error allowed 0.05

Therefore: -

= 76

Rate of PGC in Kenya is 66% of Total T2DM cases (Otieno *et al*., 2021).

Therefore: - PGC= 51, 25=GGC and healthy controls=27

Total recruited participants= 103

3.7 Data collection and laboratory procedures

3.7.1 Collection of anthropometric and demographic data

Anthropometric and demographic data of patients was collected using a well-tested data collection tool. The data include, Age, Weight, Level of education, duration of treatment, BMI, economic activity, gender, physical activity of the participant, history of familial diabetes mellitus and related complications.

3.7.2 Collection of blood samples

Blood samples for the tests were collected by a trained phlebotomist to ensure the quality of the sample and to minimize the possible risks to the participants. 6milliliters(mls) of venous blood required was placed in two different blood collection tubes as follows;3mls in EDTA purple top vacutainer, and 3mls in the red top tube with clot activator. A closed blood system method which is safer, minimize pain and prevents cross-contamination of the sample was used. In summary, the phlebotomist welcomed the participant with a duly filled study request form, the participant was requested to sit in a comfortable position, and the hand of which blood will be drawn anchored using a tourniquet. The proper vein was identified at the antecubital fossa region and the site disinfected using a 70% alcohol swab, left to dry for 30 seconds, and a venipuncture performed at a 30^0 angle. Blood was then drawn starting from the red top then followed with the purple top. The collected blood was immediately mixed gently 5 times by flip-flopping and then labeled with the participant's identifiers as per the study request form. EDTA anticoagulated blood samples were stored at 2-8^0 C as they await processing while the sample from the red top vacutainer was left to clot for about 30 minutes, centrifuged at 3000revolutions per minute for 3 minutes, and then serum is separated and placed in well-labeled serum cups then stored at 2-8^0C as it awaits processing.

3.7.3 Determination of glucose levels

Determination of diabetic status was done by measurement of blood glucose levels. All study participants were screened for blood glucose levels at the point of recruitment through rapid sugar detection tests which include the random blood sugar level and the HbA1c tests. Random blood sugar (RBS) test was done on capillary blood or venous blood collected in an EDTA tube by use of a glucometer and approximately 4 µl of sample was used. A softstyle blood glucose testing device was used for an RBS test where a test strip was placed on the RBS machine, a small blood sample taken by inserting the test strip with the device in EDTA tube with the blood sample. The results are then displayed in 5 seconds and were recorded in mmol/l as units.

HbA1c test procedure involved the following steps; 100 µl of the hemolysis buffer which was placed in a vial containing a detector diluent, 5 µl of the sample was added to the mixture in the vial and then mixed 15 times. 75 µl of the sample was then pipetted and placed on the sample window on the test cartridge. The cartridge was incubated at 30^0 C for 12 minutes then placed on the machine to read the results. The test took 5 minutes and the results were out on display and recorded. The instrument used was the Ichroma II immunolyzer which uses fluorescent sandwich immunodetection technique. The normal range for HbA1c is 4.5-6.5%.

3.7.4 Quantitative determination of renal function.

The biomarkers markers for the renal decline (creatinine, urea and calcium) were measured by an automated biochemistry analyzer, Humastar 300SR from Human Biochemical and diagnostics company. Approximately, serum sample of 500 µl was placed in a tube. Samples details were then entered on the sample list on the analyzer's monitor and the tests to be done on the sample selected. The analyzer was then loaded with the calibration and quality control (QC) material which run concurrently with the test samples. The calibrator, QC materials, and the test samples were then

loaded at the respective sample positions on the sample tray and then the machine executed to run the tests. Within 30 minutes, the results indicating completed were verified and a printout obtained. The analyzer uses a combination of spectrophometry and colorimetric methods as the main determination principles.

Electrolytes (NA^+, K^+, and CL^-) were determined through an electrometric technique using a Humalyte plus3 Ion selective electrode (ISE) analyzer. The concentrations were measured comparison to the standard solutions in the reagents and quantified. An approximate 200 µl of the sample was used. The sample was placed and the probe and aspiration key pressed, the electrolytes in the sample attracts to each specific electrode creating a membrane potential that is quantified against a constant potential at the reference electrode. The analyzer takes approximately 2 minutes to produce results which were each labeled and filed.

3.7.5 Quantitative determination of cardiac function

Creatinine kinase- MB (CK-MB) levels were determined using an immunolyzer device Ichroma II which is a point of care (POC) device which uses a fluorescent sandwich immunodetection principle. During the procedure, 10 µl of the sample was placed in respective detector diluents, mixed 10 times and 75 µl of the mixture pipetted and placed on respective test cartridges. The cartridges were then incubated for 10 minutes at room temperature and then placed on the device to be read. The normal ranges for CK-MB are between 0-7 ng/ml.

LDH procedure uses approximately 100 µl of the sample which was loaded on the Humastar analyzer, sample details were keyed in and the machine commanded to run. The test takes approximately ten minutes for the results to be displayed. The normal range of LDH is between 140 to 280 U/L.

3.7.6 Determination of Lipid Levels

Lipids profile tests include four basic parameters: total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides, and it is normally done in a fasting state blood specimens. The tests were performed using an automated clinical chemistry analyzer, Humastar 300SR. An approximate 100 µl of the serum sample was placed on the test tube and sample details loaded on the analyzer, the required tests selected, sample placed on the sample wells and the test commanded to run. Testing takes around 30 minutes for the results to be verified and printed out.

3.8 Quality assurance

All procedures from participant recruitment to sample processing and data management was conducted by well-trained professional personnel to ensure the quality of the processes. Kakamega county general hospital has a well-established diabetic clinic thus making it ideal for the study and easier follow-up procedures if need be as per the standards. Laboratory procedures were conducted in Kakamega county general hospital laboratory which is an ISO 15189-2012 accredited laboratory with highly qualified staff adhering to international quality standards. All laboratory equipment used for sample analysis were first calibrated and quality control done and passed before analysis of the samples. All results obtained were counterchecked for any errors and if need be a re-run was done to validate. Data entry was also done with double counterchecking to ensure that the data entered is equally the same as the data obtained.

3.9 Data analysis

The data collected was entered on excel, validated, cleaned and later coded and analyzed using IBM SPSS 22.0 statistics software. The normality test was done using the Kolmogorov Smirnov normality test to determine if the data is parametric or non-parametric. Sociodemographic variables were analyzed using Chi-square for categorical variables and reported in number (n) and

percentages (%) while continuous variables were analyzed using Kruskal-Wallis and reported as medians (M) and interquartile range (IQR). Analysis of hepatic, renal and cardiovascular biomarkers was done using Kruskal-Wallis and results reported as medians(m) and Interquartile range (IQR). The differences were tested at $P \leq 0.05$. A Bonferroni correction post-hoc test was done to all statistically significant results. Results were then summarized in tables.

3.10 Logistical and ethical considerations

Ethical approval for the study was sort from the MMUST institutional ethical review committee (IERC) approval number **MMUST/IERC/143/2023**, National commission for science, technology and innovation (NACOSTI) license number **NACOSTI/P/23/25518** and the Kakamega county general hospital institutional review board(IRB) approval number **ERC/200-05/2023** for data collection. Study participants had equal chance of being enrolled in the study. The participants were sensitized and taught about the importance of the study, the procedures involved in the study and what was expected from them, and the potential benefits from the study. Informed consent was sought from the participants and only consenting participants were recruited.

Each participant was assigned a specific code on the enrollment questionnaire and the samples obtained from them were linked to each participant's enrollment questionnaire code. Data obtained was electronically coded, cleaned, and stored in spreadsheets under a locked file that was only accessed by authorized personnel. The whole study followed international research code of conduct and less invasive procedures during sample collection were used to minimize harm to the participants. Any participants whose results was found to have findings that needed urgent medical attention was traced and treated as per the hospital policy and procedures. Patients had a right to withdraw from the study at any point in time without losing the rights to benefits that may come from the study.

All participants were examined by a qualified physician, and the study did not interfere with the normal treatment routine as per the Kakamega county diabetic clinic guidelines. All samples obtained were stored in appropriate storage conditions and were only accessible to authorized individuals.

CHAPTER FOUR

4.0 RESULTS

4.1 Demographic, clinical and anthropometric characteristics of study participants

A summary of the sociodemographic characteristics of the study participants is as shown in the table 4.1 below. A total of 103 adult study participants were enrolled into the study comprising of 42 males (40.8%) and 61 females (59.2%). Of these, there were healthy controls (n=27), T2DM patients with good glycemic control (HbA1c ≤7.0%) (n=25), and T2DM patients with poor glycemic control (HbA1c >7.0%) (n=51). The study participants had a mean age of 49.8 while the median age was 54 years. There was a significant difference in age (*P*= <0.0001), height (*P*=<0.0001) and weight (*P*=<0.0001) of the participants across the three groups. T2DM group with good glycemic control weighed more (Median, 83.1Kgs; IQR,19.28) than the T2DM group with poor glycemic control (Median, 73.2Kgs; IQR, 19.65) and healthy controls (Median,61Kgs; IQR, 14.0). The distribution of BMI varied across the group significantly (*P*=<0.0001) with majority of the T2DM participants being overweight (n=28; 27.18%) and Obese (n=53; 51.45%). Additionally, participants with poor glycemic control found in Obese category were more (n=18) than those with good glycemic control (n=17) and healthy group (n=1). Similarly, participants who were overweight predominated in the poor glycemic control group (n=20) than the good glycemic control group(n=6) and the healthy control group(n=1). There was also a significant variation in the hypertension status among the study group (*P*=0.028). During recruitment (n=51) of all the participants had been diagnosed with hypertension and the rest were normotensive e(n=52). The distribution across the various diabetic medication remained the same across the good glycemic control group and the poor glycemic control group, Metformin (*P*=0.298), Glibenglamide (*P*=0.222), other (*P*=0.723) and combination (*P*=0.170).

Table 4.1 Demographic, clinical and anthropometric characteristics of the study participants

Characteristic	Healthy controls n=27	T2DM (Good glycemic control n=25	T2DM (Poor glycemic control) n=51	*P*
Gender				
Male n (%)	18(66.7)	11(44.0)	13(25.5)	
Female n (%)	9(33.3)	14(56.0)	38(74.%)	**0.002**
Age, years	23(2.0)	66(13.0)	60 (18.0)	**<0.0001**
Height. Cm	172((9.0)	163.5(11.8)	160(10.5)	**<0.0001**
Weight, Kg	61(14.0)	83.1(19.3)	73.2(19.6)	**<0.0001**
BMI Kg/m^2	21.3(3.6)	31.8(10.8)	28.7(7.7)	**<0.0001**
BMI category; n (%)				
Underweight (<18.5Kg/m^2)	3(11.1)	0(0.00)	1(2.0)	
Normal (18.5-24.9kg/m^2)	21(77.8)	2(8.0)	12(23.5)	**<0.0001**
Overweight (25.0-29.9Kg/m^2)	2(7.4)	6(24.0)	20(39.2)	
Obesity (>30.0 kg/m^2)	1(3.7)	17(68.0)	18(35.3)	
Hypertension status				
Normotensive n (%)	27(100.0)	4(16.0)	21(41.2)	**0.028**
Hypertensive n (%)	0(0.00)	21(84.0)	30(58.8)	
Hypertension Categories n (%)				
New-5years	N/A	10(47.6)	14(46.7)	
6-10 years		5(23.8)	7(23.3)	**0.027**
11-15 years		3(14.3)	9(30.0)	
>15 years		3(14.3)	0(0.0)	
Period since diagnosis n (%)	N/A	3(5.0)	7(8.0)	0.061
Medication				
Metformin n (%)	N/A	18(72.0)	42(82.5)	0.298
Glibenglamide n (%)		10(40.0)	28(54.9)	0.222
Other n (%)		6(24.0)	12(23.5)	0.723
Combination n (%)		10(40.0)	32(62.7)	0.170
Period since diagnosis Category n (%)				
Newly diagnosis	N/A	2(8.0)	3(5.9)	
0-5years		15(60.0)	17(33.3)	0.203
6-10 years		4(16.0)	16(31.4)	
11-15 years		2(8.0)	10(19.6)	
>15 years		2(8.0)	5(9.8)	

Data indicates the numbers(n) and proportions (%) of the participants for the categorical variables while continuous variables presented as median and interquartile range(IQR). Chi-square test was done for the categorical proportions while Kruskal-Wallis test was done on continuous variables and significance tested at ($P \leq 0.05$). A Bonferroni post-hoc correction test was used for between groups comparisons which was set at ($P<0.0167$) and significant groups denoted as [a] ($P<0.0167$ vs Healthy controls) [b] ($P< 0.0167$ vs T2DM good glycemic control). All the statistical significant *P* values are in bold.

4.2 Hepatic Biomarkers variability across the study groups.

The summary for the hepatic biomarkers variations across the groups is summarized in the table 4.2 below. The activity of gamma glutamy transferase enzyme varied significantly across the group (*P*=0.031) with the levels increasing across the groups, healthy controls (median, 19.0; IQR, 9.10), T2DM good glycemic control (median, 24.9; IQR,17.45) and T2DM poor glycemic control (median, 26.9; IQR, 23.70). The levels of total bilirubin reduced significantly across the group(*P*=0.0001) and also the levels of direct bilirubin also varied significantly across the groups (*P*=0.0001). Additionally, levels of albumin varied significantly across the groups (*P*=0.001) and a strong difference witnessed between healthy controls (Median, 45.61; IQR 3.40) and T2DM poor glycemic control (Median, 42.71; IQR, 5.85).

The enzyme activity of Aspartate aminotransferase(AST) and alanine aminotransferase (ALT) remained the same across the groups (*P*=0.059 and *P*=0.341) respectively. However, the serum AST/ALT ratio varied significantly across the groups (*P*= 0.000). Total protein levels did not significantly vary across the three groups (*P*=0.080) with also albumin/total protein ratio not varying significantly among the participants across the groups (*P*=0.323).

Table 4.2 Hepatic biomarker variability between the healthy controls, T2DM (good glycemic control) and T2DM (poor glycemic control).

Parameter, Units(Ranges)	Healthy controls n=27	T2DM (Good glycemic control) n=25	T2DM (Poor glycemic control) n=51	*P*
AST, U/L(0-31)	22.0(9.0)	22.3(9.0)	19.3(10.0)	0.059
ALT, U/L(0-34)	17.0(10.2)	18.5(8.9)	21.3(12.2)	0.341
AST/ALT Ratio	1.27(0.6)	1.13(0.3)	0.88(0.3)[a]	**<0.0001**
Gamma-GT, U/L(0-39)	19.0(9.1)	24.9(17.5)	26.9(23.7)	**0.031**
ALP, U/L(55-105)	99.8(55.0)	68.5(51.1)	83.9(40.1)	0.076
Total Bilirubin, umol/l(1.71-20.52)	18.4(12.0)	11.9(9.2)[a]	11.3(4.9)[a]	**<0.0001**
Direct Bilirubin, umol/l(0-3.42)	6.4(7.09)	3.9(1.6)[a]	3.2(1.8)[a]	**<0.0001**
Total protein, g/l(66-87)	76.2(5.6)	73.1(5.7)	72.3(12.3)	0.080
Albumin, g/l(38-51)	45.6(3.4)	45.1(4.8)	42.7(5.9)[a]	**0.001**
Albumin/T.Protein	0.59(0.63)	0.6(0.07)	0.59(0.08)	0.323

Data shown indicates the medians and the interquartile range(IQR) for the continuous variables across the healthy controls, Type 2 diabetes mellitus (T2DM) good glycemic control and T2DM with poor glycemic control; Aspartate aminotransferase, AST; Alanine aminotransferase, ALT; gamma-GT, gamma glutamyl transferase; ALP, alkaline phosphatase. Kruskal- Wallis test was used to compare the levels across the groups at $P< 0.05$. A Bonferroni post-hoc correction test was used for between groups comparisons which was set at ($P<0.0167$) and significant groups denoted as [a] ($P<0.0167$ vs Healthy controls) [b] ($P< 0.0167$ vs T2DM good glycemic control). All the statistical significant *P* values are in bold.

4.3 Renal biomarker variability among the study participants.

The levels of urea varied significantly across the group ($P= 0.002$) with a stronger variation being between the healthy controls and the T2DM good glycemic control. The levels of electrolytes also varied significantly across the groups with potassium[K^+] ($P=0.012$), Sodium[Na^+] ($P<0.0001$) and chloride[Cl^-] ($P=0,007$). Creatinine levels did not vary significantly across the groups ($P=0.501$). eGFR levels distribution remained the same across all the groups ($P=0.241$), However, there was evident reduction across the healthy controls (median, 82.2; IQR, 18.40), T2DM good

glycemic control (median, 81.0; IQR, 29.15) and T2DM poor glycemic control (median, 71.11; IQR 32.80). The distribution of calcium levels remained the same across the study groups (*P*=0.228). The summary of the data is shown in table 4.3 below.

Table 4.3 Renal Biomarker variability between healthy controls, T2DM (good glycemic control and T2DM (poor glycemic control).

Parameter, units(Ranges)	Healthy controls n=27	T2DM (Good glycemic control) n=25	T2DM (Poor glycemic control) n=51	*P*
Creatinine, umol/l(53.93-99.01)	96.5(25.6)	103.0(38.0)	100.4(30.5)	0.501
Urea, mmol/l(1,83-7.5)	3.4(1.2)	4.8(2.5)[a]	3.8(1.4)	**0.002**
Potassium[K^+], mmol/l(3.5-5.5)	4.02(0.49)	4.12(0.55)	4.29(2.15)	**0.012**
Sodium[Na^+], mmol/l(135-145)	145.8(2.00)	144.0(3.10)	142.02(4.20)[a]	**<0.0001**
Chloride[Cl^-], mmol/l(98-107)	106.9(3.80)	110.3(7.55)[a]	106.4(7.70)[b]	**0.007**
eGFR (≥90)	92.2(18.40)	87.0(29.15)	85.11(32.80)	0.241
Calcium, mmol/l(2.02-2.6)	2.25(0.41)	2.42(0.48)	2.32(0.55)	0.228

Data shown indicates the medians and the interquartile range(IQR) for the continuous variables across the healthy controls, Type 2 diabetes mellitus (T2DM) good glycemic control and T2DM with poor glycemic control; eGFR, glomerular filtration rate; Kruskal- Wallis test was used to compare the levels across the groups at *P*< 0.05. A Bonferroni post-hoc correction test was used for between groups comparisons which was set at (*P*<0.0167) and significant groups denoted as [a] (*P*<0.0167 vs Healthy controls) [b] (*P*< 0.0167 vs T2DM good glycemic control). All the statistical significant *P* values are in bold

4.4 Cardiovascular biomarkers variability among the study participants

The cardiovascular biomarkers data is summarized in the table 4.4 below. Triglycerides levels varied significantly(*P*=0.0001) across the groups. The significant variation was also witnessed with total cholesterol levels(*P*=0.046) with the levels also increasing across the Healthy control (median, 4.00; IQR, 1.17), T2DM good glycemic control (median, 4.78; IQR, 1.48) and T2DM poor glycemic control (median, 5.00; IQR, 1.67). LDH level distribution also significantly varied

across the group(P=0.005) with an increase witnessed between the healthy control group and the diabetic group with good glycemic control and poor glycemic control group.

The distribution of the CK-MB levels was not statistically significant across the groups(P=0.961). Similarly, the distribution remained the same(P=0.709) with the HDL levels across the study groups even though there was an increase in the levels across the groups.

Table 4.4 Cardiovascular biomarkers variability between healthy controls, T2DM (good glycemic control and T2DM (poor glycemic control).

Parameter, units(Ranges)	Healthy control n=27	T2DM (Good glycemic control) n=25	T2DM (Poor glycemic control) n=51	*P*
HDL, mmol/l(1,16-1.55)	1.42(0.40)	1.33(0.42)	1.30(0.60)	0.709
LDL, mmol/l(0-2.59)	2.80(1.40)	2.85(1.06)	2.90(1.31)	0.961
Triglycerides, mmol/l(0.4-1.54)	1.0(0.15)	1.94(1.36)[a]	2.05(3.31)[a,b]	**<0.0001**
Total-cholesterol, mmol/l(0-4.91)	4.00(1.17)	4.78(1.48)	5.00(1.67)[a,b]	**0.046**
CK-MB, ng/ml(0-7)	3.00(1.53)	3.00(1.53)	3.00(1.02)	0.961
LDH, U/l(225-450)	334.73(87.)	417.1(147.28)[a]	405.98(134.33)[a]	**0.005**

Data shown indicates the medians and the interquartile range(IQR) for the continuous variables across the healthy controls, Type 2 diabetes mellitus (T2DM) good glycemic control and T2DM with poor glycemic control; HDL, High density lipoprotein; LDL, low density lipoprotein, CK-MB, Creatine-kinase MB isoenzyme; LDH, lactate dehydrogenase; Kruskal- Wallis test was used to compare the levels across the groups at P< 0.05. A Bonferroni post-hoc correction test was used for between groups comparisons which was set at (P<0.0167) and significant groups denoted as [a] (P<0.0167 vs Healthy controls) [b] (P< 0.0167 vs T2DM good glycemic control). All the statistical significant P values are in bold.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Demographic and anthropometric characteristics of T2DM

The study found out that more women had poor glycemic control as compared to their male counterparts. This may be attributed to adiposity differences where women have more lipogenesis and less fatty acids turnover as compared to males and differences in physical activities which plays a role in increasing insulin sensitivity (Kautzky-Willer *et al*., 2016; Mamo *et al*., 2019; Wagai & Romshoo, 2020). Socio-economic differences also play a role in these differences since most women are perceived to have less social support, more stressed, poor nutritional habits and also likely to be involved in polypharmacy which increases drug-drug interaction reducing diabetic therapy efficacy (Dobrică *et al*., 2019). These findings are in line with a study conducted in Korea which suggests that women are less likely to achieve glycemic control targets when initiated to treatment as compared to males (Choe *et al*., 2018). A study conducted in 24 countries also suggested that women had poor glycemic control and response to treatment as compared to males (Ferrannini *et al*., 2021).With these findings, a gender based approach should be considered during treatment and management of T2DM.

Poor glycemic control was observed in younger age group. This may be associated to several factors including poor medication adherence, lack of self-glucose monitoring devices, dyslipidemia, obesity and high smoking rates among young patients (Mamo *et al*., 2019; Nanayakkara *et al*., 2018). This finding correlates with a study conducted by S. M. Shamshirgaran in Iran which indicated that older patients were less likely to develop poor glycemic control as compared to young diabetic patients (Shamshirgaran *et al.*, 2017). Another study conducted in

Australia indicates that younger patients with T2DM were associated with poor glycemic control and an increased likelihood of developing related complications (Nanayakkara *et al*., 2018).

Low BMI was observed in non-diabetic control group as compared to T2DM group. This finding may be attributed to a lower age of healthy control group. However, diabetes being a metabolic disorder, leads to poor metabolism of fats, causing increased lipogenesis and low fatty acids turnover in diabetes patients thereby affecting adiposity (Mamo *et al*., 2019; Wagai & Romshoo, 2020). Obesity has a direct link in development of diabetes through increase of non-esterified fatty acids and glycerol, hormonal imbalances and overproduction of inflammatory cytokines. These factors affect the β-islets cells leading to low insulin production and overall insulin insensitivity (Al-Goblan *et al*., 2014). The finding is in line with a similar study reporting high levels of BMI in diabetes mellitus (Veit *et al*., 2022). The study findings further revealed that individuals with good glycemic control had more weight, height and consequently a high BMI as compared to participants with poor glycemic control. This finding may be attributed to an older age among patients with good glycemic control as compared to those with poor glycemic control. This finding can be associated to factors such as smoking and physical inactivity. Even though not covered in this study, these factors contribute to increased insulin insensitivity, and fatty acids metabolic disturbances leading to increased peripheral fatty acids and triglycerides deposition (Mamo *et al*., 2019). Similar findings have been reported with other studies conducted on diabetic risk factors among T2DM patients (Hassan *et al*., 2021; Muringo *et al*., 2021).

Hypertension was significantly common among individuals with good glycemic control compared to T2DM patients with poor glycemic control. This can be associated with hypertension risk factors such as old age and increased physical inactivity. Such factors play a role in reducing insulin response to glucose and increase of inflammation which results to poor lipid metabolism, this leads

to lipids accumulation in the vasculature narrowing the lumen thus increasing resistance to blood flow (Mamo *et al*., 2019). Contrary to this finding, other studies indicate that individuals with poor glycemic control are prone to hypertension. The differences could be as a result of variability in risk factors with respect to different weights, height and ages (Mariye *et al*., 2019; Muringo *et al*., 2021).

The period since initial diagnosis varied insignificantly with individuals with poor glycemic control having more years as compared to individuals with good glycemic control. This could be due to progressive impairment of the β-pancreatic cells that occurs with time (Shita & Iyasu, 2022). Other studies also reveals a non-significant association of poor glycemic control and period since diagnosis (Nigussie *et al*., 2021).

5.2 Hepatic biomarker variability between the study groups

The study results revealed no significant different in the levels of alanine aminotransferase (ALT) across the study groups indicating no hepatocellular damage in poor glycemic control participants. T2DM state usually affects metabolic pathways of various molecules in the liver, such disturbances lead to accumulation fatty acids in the hepatocellular spaces and increased oxidative stress which increases pressure on the cells leading to their damage. (Islam *et al*., 2020; Maritim *et al*., 2003; Noroozi Karimabad *et al*., 2022). Poor glycemic control elevates these factors leading to an increased hepatic damage. The fat deposition leads to non-alcoholic fatty liver disease(NAFLD) whose features include microvascular hepatocellular steatosis resulting in Liver enzymes elevation (Mertens *et al*., 2021; Shahwan et al., 2019). Similar to this study, a study conducted by Saligram *et al* indicates slight increase in ALT levels within the physiological range. They concluded that these slight differences are due to weight and lipids rather than glycemic control (Saligram *et al.*, 2012).

Contrary to these findings, other studies have reported elevation of ALT levels in poor glycemic control (Islam *et al*., 2020; Nwosu *et al.,* 2012).

In addition, levels of aspartate aminotransferase (AST) reduced in individuals with poor glycemic control as compared to individuals with good glycemic control. This may be attributed to the higher weight in individuals with good glycemic control. Since AST is non-specific to the liver, the elevation maybe due to other diabetic increased factors such as weight and muscle mass which are not directly related to the liver and glycemic control (Saligram *et al.,* 2012). These findings differ with other studies done which indicate elevation of AST due to liver damage in T2DM (Islam *et al*., 2020; Noroozi Karimabad *et al*., 2022; Shahwan *et al*., 2019).

The AST/ALT ratio reduced significantly across the study groups with good glycemic control having a slightly higher ratio as compared to the poor glycemic control. A possible reason is the inverse relationship witnessed in the levels of individual AST and ALT levels. The reason for this inverse relationship remains unclear, considering that many other study indicates that both the enzymes elevate in case of the hepatocellular damage of any kind (Noroozi Karimabad *et al*., 2022).

The study also revealed an increase in the median levels of γ-glutamyl transferase (GGT) within the physiological range in patents with T2DM as compared to non-diabetics/Healthy controls. Additionally, T2DM patients with poor glycemic control had higher GGT levels as compared to the group with good glycemic control. The Higher levels can be due to two factors. Firstly, diabetes causes lipid metabolism disturbances, this leads to increased fat deposition in tissues among them the liver cells which consequentially may lead to non-alcoholic fatty liver disease (NAFLD). Such causes pressure to the hepatic cells in this case the cells lining the hepatobiliary system known to produce the GGT enzyme. This leads to increased production of the enzyme by these cells (Islam *et*

al., 2020; Williams *et al*., 2013). Secondly, diabetes is known to induce oxidative stress via increased production of superoxide and ROS molecules that affects the cell membrane (Giacco & Brownlee, 2010). GGT is a transmembrane enzyme and also serves as an anti-oxidant, an increase in the oxidative stress as in the case of T2DM with poor glycemic control results to increase GGT enzyme production(Gohel & Chacko, 2013). Other studies have also reported the same elevation of the enzyme GGT due to diabetes mellitus with more increase being witnessed in poor glycemic control patients (Gohel & Chacko, 2013; Islam *et al*., 2020).

The results also showed a significant reduction in the levels of direct bilirubin and total bilirubin across the study groups, with healthy controls having higher levels than the good glycemic control group and subsequently the poor glycemic control group having the least. Bilirubin levels are inversely associated with metabolic syndrome (Zhu *et al*., 2017). Low bilirubin levels in the body are also considered as a risk factor for development of diabetes and diabetes related complications(Yang *et al*., 2019). Diabetes is a metabolic disorder which leads to increased oxidative stress. On the other hand, bilirubin is a natural antioxidant thus preventing the cells against diabetic related metabolic deleterious effects on the cells (Fu et al., 2010). The results in this study were in agreement with a study conducted by Erkus *et al* in 2017 which indicated that low levels of bilirubin were associated with poor glycemic control (Erkus *et al*., 2018). These findings thus strongly suggest the use of bilirubin levels as potential biomarker for glycemic control.

The median levels of total proteins were lower in diabetic group as compared to health control group. However, there was no statistical difference in this levels between the groups indicating minimal effect on hepatic protein synthetic function in T2DM. In slight reduction in median levels in T2DM may be due to an increased proteins glycation which results in formation of various

products such as AGEs through a non-enzymatic condensation of amino acids residues and the reducing sugars leading to irreversible structural reorganization of the end products (Twarda-Clapa *et al*., 2022). These glycated proteins have a shorter half-life than normal protein molecules and they are prone to proteasomal degradation making the total protein levels slightly lower in diabetes and also in poorly controlled diabetes mellitus (Raupbach *et al*., 2020). A study conducted in Ghana in 2019 revealed that poor glycemic control was associated with a reduction in the levels and activities of plasma proteins the results of which are in line with the findings of this study (Addai-Mensah *et al*., 2019). However, the study in Ghana only focused on three plasma proteins; Protein C(PC), Protein S(PS) and antithrombin III (AT III). Contrary to these findings, a study conducted in Nigeria in 2019 revealed a higher levels of total proteins in T2DM patients as compared to the healthy control group the results which correlates with another study conducted in India in 2020 (Analike *et al*., 2019; Kumari & Supriya, 2020).

5.3 Renal biomarker variability between the study groups.

There was no statistical difference in creatinine levels across the study groups which indicate that poor glycemic control had minimal effects on the renal glomerulus. However, the median values were slightly lower in the poor glycemic control group as compared to good glycemic control. Diabetes is known to cause Diabetic Nephropathy where their destruction of the glomerulus due to oxidative stress, increased pressure and increased AGEs causing thickening of the glomerulus basal membrane leading to failure of the kidneys to filter out metabolic wastes creatinine being one of them (Duran-Salgado & Rubio-Guerra, 2014; Lim, 2014). These results are in contrast with results from another study done in Japan which indicate that creatinine levels are significantly lower in diabetes as compared to healthy non-diabetic individuals (Harita *et al*., 2009). Contrary to these findings, a study conducted by Faheem and others revealed that creatinine levels were higher

in poor glycemic control as compared to good glycemic control(Faheem *et al*., 2019). The differences in the results may be due to differences in ethnicity which plays a role in variation of creatinine levels. Therefore, with the variability in the findings, Creatinine alone should not be used as a sole indicator of diabetes related kidney disease but instead, other renal markers should be measured and compared.

A significant high levels of urea among the T2DM patients was recorded in the study as compared to healthy controls. Additionally, among the diabetic group, patients with good glycemic control had higher urea levels than patients with poor glycemic control. These results suggest that T2DM is associated with mild azotemia even though the increase is within the normal range. Urea is the main waste formed from the metabolic breakdown of proteins in the liver and is transported to the kidneys for excretion (Higgins, 2016). T2DM increases protein glycosylation which in turn leads to increased glycation products in the body (Clerc *et al*., 2016). The reaction causes alteration of the proteins which becomes candidates for proteolytic and enzymatic breakdown in the liver (Raupbach *et al*., 2020). High levels of urea is known to be a risk factor for T2DM where it increases insulin secretory defects, impairs glycolysis by increasing the islets protein *O*-GlcNAcylation (Al-Musawi *et al*., 2021; Koppe *et al*., 2016). Similar to the findings of this study, is a study conducted by Dutta *et al* suggesting ammonia and urea levels elevation in Diabetes condition as compared to the non-diabetics (Dutta *et al*., 2016). Contrary to the findings, a study conducted by Faheem and others reports poor glycemic control is characterized with increased urea levels which increases the chances of developing diabetic nephropathy as compared to good glycemic control (Faheem *et al*., 2019).

The study findings revealed a significant difference in the levels of potassium(K^+) in T2DM patients as compared to the healthy control. Even though the values remain to be within the normal

range, diabetes is usually known to cause variations in electrolyte balance. These imbalances can further lead to other complications such as cardiovascular, and neuromuscular diseases (Pipeleers *et al*., 2019). The variation witnessed in the study may be brought about via various mechanisms which include compromised K^+ shift back into the cells and impaired excretion due to a deranged tubular function system (Goia-Nishide *et al*., 2022). Diabetes mellitus also leads to a condition known as Hyporeninemic hypoaldosteronism a condition that results in a defective RAAS system which thus leads to reduced Na^+ reabsorption and secretion of K^+ into the urinary excretion ducts. This in turn leads to an increase in blood potassium levels (Sousa *et al*., 2016). Additionally, increased plasma osmolality especially in poor glycemic control leads to water shifting out of the cells. To maintain the balance and revert to the normal state, an opposite electrolyte shift occurs leading to more K^+ ions being pumped outside the cells leading to their increased concentrations (Adeyinka & Kondamudi, 2022; Goia-Nishide *et al*., 2022). In line with these findings, other studies also indicate significant variation in potassium in Diabetes mellitus which is may be elevated in uncontrolled hyperglycemia(Goia-Nishide *et al.*, 2022). Contrary to the findings of this study, some study indicate that DM leads to hypokalemia especially when there is strong use of diuretics, insulin, beta-2 agonists, antiarrhythmic agents, laxatives and use of glucocorticoids and mineralocorticoids (Coregliano-Ring *et al*., 2022). Therefore, in this case the hypokalemia is not directly attributed to hypeglycemia but rather to the use of these treatment agents. Whereas this study reported a significant difference in potassium levels between good and poor glycemic control T2DM patients, a study conducted in Indian T2DM patients by Karuppan reports no significance difference in K^+ levels between controlled and uncontrolled diabetes (Karuppan *et al*., 2019).

The study reported lower levels of sodium (Na^+) in T2DM patients. Glucose is a metabolically active substance which means it draws water towards it. High glucose levels as witnessed in

diabetes causes a directional water flow out of the cells thus over diluting the extracellular milieu. This dilution mechanism subsequently lowers the levels of sodium in blood. This unidirectional flow of water however does not cause edema because reasons such us increased urine output ,increased fluids loss, and lack of protein leakage (Liamis et al., 2014). Uncontrolled diabetes can also lead to hypovolemic hyponatremia via induced diuresis. The results of these study correlate with other studies which also report low levels of sodium in T2DM and poorly controlled T2DM (Khan *et al*., 2019). Contrary findings were reported by Engwa and others in a study conducted in Nigeria which suggested that sodium levels increased in T2DM patients as compared to non-diabetic patients (Engwa *et al*., 2018). A study conducted among the Chinese population also revealed that serum sodium levels were significantly decreased in diabetic patients. However the decrease was more significant in diabetic patients with normal glucose regulation as compared to those impaired glucose regulation (Wang *et al*., 2013).

The study reported a significant difference in chloride (Cl^-) levels between T2DM patients with good glycemic control and those with poor glycemic control. The levels of chloride were low in poorly controlled DM as compared to good glycemic control. Serum chloride levels are affected by the balance in other electrolytes especially sodium with the main mechanism leading to hypochloremia being as a result of increased osmolality due to high glucose levels leading to extracellular chloride dilution due to high water movement outside the cells (Khan *et al*., 2019). Similar findings were reported in India where among 342 study participants, 31% had hypochloremia and it was significantly common among subjects with uncontrolled DM (Karuppan *et al*., 2019).

The serum calcium levels were insignificantly low among the poor glycemic control T2DM patients as compared to the controlled glycemic control group. Diabetic condition leads to

derangement in calcium homeostasis via various mechanisms such as hypovitaminosis D, interference with Calcium-regulating hormones such as Parathyroid hormone(PTH),1,25 dihydroxyvitamin D and also impaired function of calcium regulating organs which causes impaired intestinal calcium absorption, bone resorption and or deterioration and renal calcium wasting (Wongdee et al., 2017). Contrary to these findings, Wang et al reports that calcium levels increase with increased glucose levels among the Chinese diabetic patients (S. Wang *et al*., 2013).

Serum electrolyte measurement remain essential biomarker for determining renal function and related complications in T2DM. However, due to differences in mechanisms leading to the variability as seen in this study and various other studies, their clinical use and interpretation should be coupled with clear clinical history, association with other biomarkers and presentation of the patient.

5.4 Cardiovascular Biomarker variability between the study group

Triglycerides levels were significantly high in diabetic group with poor glycemic control as compared to the group with good glycemic control. Several studies suggests the importance of serum triglycerides determination since is a more useful independent predictor of atherosclerotic cardiovascular disease in people with T2DM (Hirano, 2018). Impaired/ abnormal triglycerides levels can be attributed to various mechanisms which occur concurrently in diabetes state. Firstly, increased insulin insensitivity which leads to compensatory hyperinsulinemia elevated VLDL-TG secretion. Secondly, absolute insulin deficiency leading to increased hepatic secretion of VLDL-TG and Lastly, severe chylomicronemia which is common in T2DM due to deficiency in insulin dependent lipoprotein lipase (Hirano, 2018; Yanai *et al*., 2019). In line with these findings, a study conducted in Iran among T2DM patients also reported an increase in triglycerides levels in poorly regulated glycemic condition(Mostofizadeh *et al*., 2019). Similar to these findings, a study done in

china to determine the relationship between the lipid profile and glycemic control in T2DM patients revealed high levels of triglycerides among poorly controlled sugar levels(S. Wang *et al.*, 2020).

There was no significant difference in the levels of high density lipoprotein across the study groups which indicates low risk of cardiovascular disease among the study participants. HDL-C levels are usually known to exist inversely with the levels of TGs. Low levels have been reported to be independent predictor of cardiovascular disease(CVD) in diabetes (Femlak *et al*., 2017; He et al., 2018). T2DM causes an increase in plasma TGs which results to HDL-C increased catabolism via a mechanism dependent on hepatic lipase and cholesteryl ester transfer protein (CETP). CETP transfers the cholesterol esters from HDL to triglyceride-rich lipoprotein(TGR-LPs) in exchange for TGs leading to generation of HDLs that are a substrate for hydrolysis by hepatic lipase which consequently results in their reduced plasma levels (Barter, 2011; He *et al*., 2018).The reduction of HDL-C is also coupled with a reduction in Apolipoprotein A-1(ApoA-1) (Rhee *et al.*, 2017). Reduced HDL levels results to low endothelial protection against leucocytes infiltration a result of elevated LDL-C levels and also leads reduced insulin secretion (Vaisar *et al*., 2018; Xepapadaki *et al*., 2021). Poor glycemic control association with low HDL-C has also been reported in other similar studies (AL-Bahrani & Yassin, 2022; S. Wang *et al*., 2020).

Additionally, low density lipoprotein(LDL-C) levels remained the same across the study groups. This finding indicates reduced risk of atherosclerosis other cardiovascular diseases that usually occurs due to persistent deposition of LDL-C in the vasculature (Mundi *et al.*, 2017). Insulin resistance in T2DM results in hypertriglyceridemia which causes an increase in the release of triglyceride-rich VLDL by the liver, the compound is then hydrolyzed by lipoprotein lipases to form LDL that can later form sdLDL via the action of CETP and hepatic lipase lipolysis (Adiels *et*

al., 2008). The study findings are in line with other studies which report an elevation in the levels of LDL especially in poorly controlled glycaemia(Mostofizadeh *et al*., 2019; S. Wang *et al*., 2020).

Total cholesterol levels were significantly high in diabetic group with poor glycemic control as compared to the group with good glycemic control. This is an indication of poor lipid metabolic control similar to that of TGs (Wu & Parhofer, 2014). In line with these findings, a study conducted in Iran among T1DM patients also reported an increase in total cholesterol levels in poorly regulated glycemic condition. Similar results indicating dyslipidemia among poorly controlled diabetes was also reported in a study conducted in Iraq in 2020 (AL-Bahrani & Yassin, 2022; Mostofizadeh *et al*., 2019).

Compared to the control group, Lactate dehydrogenase(LDH) levels where high among T2DM participants. These results are comparable with other studies which report increased serum LDH levels among Diabetic patients(Dmour *et al*., 2020). LDH enzyme is usually cytosol bound, T2DM cause its increased serum activity by increased damage to the cells and tissues via mechanisms such as inflammation, autoimmune cells destruction, increased AGEs formation, increased leucocytes infiltration due to the damage and deposition of lipids in blood vessels, hypoxia and oxidative stress (Archundia Herrera *et al*., 2017; Malicka *et al*., 2016; Mundi *et al*., 2017; Twarda-Clapa *et al*., 2022). However, the study reported a reduced level of LDH among the poorly controlled T2DM patients as compared to participants with good glycemic control. Studies however suggest otherwise with some reporting that LDH levels significantly increases with increase in HbA1c levels thus an indicator of uncontrolled glycemic control(Hsieh *et al*., 2022). The possible explanation to this variation is the fact that, LDH is highly affected by age(Al-Musawi *et al*., 2021), study results indicate that majority of participants with good glycemic control had a higher age as compared to participants with poor glycemic control.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The study indicates that AST/ALT ratio, gamma-GT and albumin increased significantly while Total bilirubin and direct bilirubin decreased in poor glycemic control state. This thus indicate that

a constant state of hyperglycemia due to poor glycemic control causes hepatic damage which may be fatal if left unregulated for a long time.

The levels of urea and chloride increased significantly while sodium levels reduced in poor glycemic control. This shows that there is an alteration in renal glomerular and tubular function. Increased protein turnover is a major cause of acute elevation in urea levels as compared to glomerular damage.

The levels of triglycerides and total cholesterol were significantly increased in poor glycemic control. Thus this indicate that poor glycemic control in T2DM causes dyslipidemia which thus may affect the cardiovascular functioning.

6.2 Recommendations for action

Biomarkers for renal function should be part of essential routine tests during every diabetic clinic visits and monitoring done at every stage of the management process.

Laboratory monitoring of hepatic biomarkers remains a critical part in the management of diabetes mellitus. Therefore, a liver biomarker panel testing should be incorporated as a routine investigation upon diagnosis, during treatment monitoring and in all cases of poor glycemic control.

Investigation of lipid profile should be an essential part of the routine laboratory checkup since they are significantly affected by poor glycemic control. Management should be tailored towards the regulation of sugar levels together with the regulation of dyslipidemia.

The use of these biomarkers in association with HbA1c levels, RBS coupled with sociodemographic characteristics of the patients should be routinely be utilized in clinical practice for early detection of poor glycemic control and related complications which will thus have an

effect in improvement of healthcare management, improve the disease outcomes which will ease the health economic burden.

6.3 Recommendations for further research

Similar studies with additional biomarkers should be conducted to supplement the routine biomarkers studied in this study.

Long longitudinal studies to be conducted to determine the progressive biomarker variability in hepatic, renal and cardiovascular damage.

Studies involving associations, sensitivity and specificity of these biomarkers among the type 2 diabetes mellitus.

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APPENDICES

Appendix I: Consent form (English version)

INFORMED CONCENT FOR PARTICIPANTS (filled by research participants)

CODE NO……

Title of the study: HEPATIC, RENAL AND CARDIOVASCULAR BIOMARKER VARIABILITY AMONG TYPE 2 DIABETES MELLITUS PATIENTS WITH POOR GLYCEMIC CONTROL

Investigator: Kevin Soita

Phone: 0707187185/**0796387389**

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P.O BOX 190-50100, Kakamega, Kenya.

Study Description/Purpose of the study: The study aims at determining various biomarkers of renal and cardiovascular damage, making it easier for early detection of diabetes related complications, prediction of these complications thus providing an early intervention and selecting an appropriate treatment option thus improving care, minimizing management costs and improving the quality of life.

Procedures: Blood samples will be needed from the participants. A small venipuncture will be done using a needle and 6mls of blood drawn. The procedure will take approximately 10 minutes and will be conducted by a trained Phlebotomist.

Benefits: There will be no direct benefits from the study, however, results obtained from this study will help in improving Diabetic care and management. Any future benefits that may arise will be communicated to all participants appropriately.

Risks: Any risks involved with the procedure for drawing blood sample will be minimized by ensuring that there is proper sample collection using a trained phlebotomist. Any discomfort and concern that may arise during and after the procedure should be reported immediately. Could there be any statistic i.e. critical value that need immediate patient attention, the participant will be informed and an appropriate intervention taken by a medical professional.

Confidentiality: Statement: information obtained from the participants will be purposefully used for this study alone. This information will not be shard to any third party without approval from the study participants.

Right to Withdraw: the participant has a right to withdraw from the study at any point in time and will not risk losing any of the benefits and will continue with the management and treatment as per the Hospital management guidelines.

Right for legal action: The researcher has the right to place any legal action if they feel like any the study has violated their constitutional rights and any other ethical violations during the study period.

Comments and
suggestions……
……
……
……

Declaration
Having actively participated in the filling of this consent and confirmed that the same is true on paper, I willingly sign.

Printed Name of participant..... Date.....

Signature of Principal Investigator …..... ……. Date.....

Note: By putting your signature, you are agreeing that: the questionnaire has been filled with you actively participating and have been given the opportunity to ask questions.

Investigator/ research Assistant

Signature……. ….. Date…….

The participant received copy

THANK YOU FOR AVAILING YOUR TIME AND INPUT TO THIS STUDY.

Appendix II: Consent form (Swahili version)

TAARIFA ILIYOFAHAMIKA KWA WASHIRIKI (iliyojazwa na washiriki wa utafiti)

CODE NO……...

Kichwa cha utafiti: AINA YA BIOMARKERS ILIYOCHAGULIWA MIONGONI MWA WAGONJWA WA AINA YA 2 WA KISUKARI WENYE UDHIBITI MBOVU WA SUKARI.

Mpelelezi: Kevin soita

Nambari ya simu: 0707187185/0732412827

Barua Pepe: kevinsoita67@gmail.com

Masinde Muliro University of Science and Technology

P.O BOX 190-50100, Kakamega, Kenya.

Maelezo/Madhumuni ya Utafiti: Utafiti unalenga kubainisha viashirio mbalimbali vya uharibifu wa figo na moyo na mishipa, ili kurahisisha utambuzi wa mapema wa matatizo yanayohusiana na kisukari, utabiri wa matatizo haya hivyo kutoa uingiliaji wa mapema na kuchagua chaguo sahihi la matibabu hivyo kuboresha huduma. , kupunguza gharama za usimamizi na kuboresha ubora wa maisha.

Taratibu: Sampuli za damu zitahitajika kutoka kwa washiriki. Kutoboa kidogo kutafanywa kwa kutumia sindano na 6mls ya damu itatolewa. Utaratibu utachukua takriban dakika 10 na utafanywa na Phlebotomist aliyefunzwa.

Manufaa: Hakutakuwa na manufaa ya moja kwa moja kutoka kwa utafiti, hata hivyo, matokeo yaliyopatikana kutoka kwa utafiti huu yatasaidia katika kuboresha utunzaji na usimamizi wa Kisukari. Manufaa yoyote yajayo ambayo yanaweza kutokea yatawasilishwa kwa washiriki wote ipasavyo.

Hatari: Hatari zozote zinazohusika na utaratibu wa kuchora sampuli ya damu zitapunguzwa kwa kuhakikisha kuwa kuna mkusanyiko unaofaa wa sampuli kwa kutumia phlebotomist aliyefunzwa. Usumbufu na wasiwasi wowote unaoweza kutokea wakati na baada ya utaratibu unapaswa kuripotiwa mara moja. Je, kunaweza kuwa na takwimu yoyote kama vile thamani muhimu inayohitaji uangalizi wa haraka wa mgonjwa, mshiriki ataarifiwa na uingiliaji kati unaofaa kuchukuliwa na mtaalamu wa matibabu.

Usiri: Taarifa zitakazopatikana kutoka kwa washiriki zitatumika kimakusudi kwa utafiti huu pekee. Taarifa hii haitakuwa wazi kwa wahusika wengine bila idhini kutoka kwa washiriki wa utafiti.

Haki ya Kujitoa: mshiriki ana haki ya kujiondoa kwenye utafiti wakati wowote na hatahatarisha kupoteza manufaa yoyote na ataendelea na usimamizi na matibabu kulingana na miongozo ya usimamizi wa Hospitali.

Haki ya kuchukuliwa hatua za kisheria: Mtafiti ana haki ya kuchukua hatua yoyote ya kisheria ikiwa anahisi kama utafiti wowote umekiuka haki zao za kikatiba na ukiukaji wowote wa kimaadili katika kipindi cha utafiti.

Maoni na
mapendekezo .....
.....
.....
.....

Tamko

Kwa kuwa nimeshiriki kikamilifu katika kujaza kibali hiki na kuthibitisha kuwa hiyo ni kweli kwenye karatasi, naitia saini kwa hiari.

Jina la Mhusika ……. Sahihi……...

Saini ya Mpelelezi Mkuu.....Tarehe.....

Kumbuka: Kwa kuweka sahihi yako, unakubali kwamba: dodoso limejazwa na wewe kushiriki kikamilifu na umepewa fursa ya kuuliza maswali.

Mshiriki alipokea nakala

ASANTE KWA KUPATA MUDA WAKO KUSHIRIKI KATIKA UTAFITI HUU.

Appendix III: Laboratory Request Form

HEPATIC RENAL AND CARIOVASCULAR BIOMARER VARIABILITY IN TYPE 2 DIABETES MELLITUS PATIENTS WITH POOR GLYCEMIC CONTROL

LABORATORY REQUEST FORM

Participants details **Medication**

Unique Code……...
Age…….
Gender M……...F……
Longevity (yrs)……
Lab No……...

Rx……
……
Date……

Date of sample collection……
Time of sample collection……

Participants Vitals
Height…….....
Weight……..
HWR……..MUAC……..

Sample Type; Tick appropriately

Blood ☐ Swab ☐ Urine ☐
Other…….

Relevant clinical information……

Investigation Required

CFT				**RFTS**		**SEROLOGY**	
Troponin I	☐	LDH	☐	Creatinine	☐	HBSag	☐
CK-MB	☐	AST	☐	Urea	☐	HCV	☐
BNP	☐			Electrolytes	☐	HAV	☐

Lipid profile		**OTHERS**	
Triglycerides	☐	Random blood sugar	☐
T. Cholesterol	☐	HbA1c	☐
HDL-Cholesterol	☐	LFT	☐
LDL-Cholesterol	☐	microalbumin	☐

NOTE: THIS REQUEST FORM IS ONLY INTENTED FOR THIS STUDY ALONE AND SHOULD NOT BE USED AS A SUBSTITUTE FOR THE FACILITY REQUEST FOR

Appendix IV: Data collection tool

Title: SELECTED BIOMARKERS VARIABILITY AMONG TYPE 2 DIABETES MELLITUS PATIENTS WITH POOR GLYCEMIC CONTROL

Study participant code ……. Study participant's residence…….

Interview date ……. /……. /20….

1. **Age** ……(Years) **Birth date** ……. /……./…….
2. **Gender**: Male ☐ Female ☐
3. **Weight** ……Kgs, **Height**…….cm, **Waist**……...cm
4. **Mid upper arm circumference**……cm, **Hips circumference**…….cm
5. **Religion**: None ☐ Catholic ☐

Protestant ☐ Muslim ☐

6. **Which sub county in Kakamega do you reside in?**

Lugari ☐

Likuyani ☐

Malava ☐

Lurambi ☐

Shinyalu ☐

Ikolomani ☐

Butere ☐

Matungu ☐

Mumias East ☐

Mumias West ☐

Khwisero ☐

Navakholo ☐

7. **Education Level;** None ☐ Primary ☐ Secondary ☐ Tertiary ☐
8. **Marital Status**; Single ☐ Married ☐ Divorced ☐ Widowed ☐ separated ☐
9. **Employment status**; Unemployed ☐ Self-employed ☐ Employed ☐
10. What is your income range per month?.....
11. Do you take alcohol? Yes ☐ No ☐
12. When were you first diagnosed with diabetes? Specify time in years/months……
13. Are you on antidiabetic drugs? Yes ☐ No ☐
    a) If Yes, For How long

0-6 months ☐ 6-12months ☐ 1-3years ☐ 4-6years ☐ 7-9years ☐ above 10years ☐

    b) Have you ever switched between different antidiabetic drugs? Yes ☐ No ☐

What was the reason? …….

14. Apart from antidiabetic drugs, Are you on any other long-term medication? Yes ☐ No ☐
    a) If yes, what condition are the medication meant to treat ……
15. Do you have anyone in the family with a Heart disease? Yes ☐ No ☐
16. Do you have anyone in the family with a kidney disease? Yes ☐ No ☐

17. Have you ever had a liver or kidney transplant? Yes ☐ No ☐

18. Do you have a personal blood sugar testing device? Yes ☐ No ☐

19. How many times do you visit a health facility in a year?

    1-3 ☐ 4-6 ☐ 7-9 ☐ 10- above ☐

20. Are you currently suffering from any of the conditions listed before? Please tick appropriately

    Tuberculosis ☐ HIV/AIDS☐ Hypertension☐ Renal disease☐

Appendix V: School of Graduate studies approval

MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY (MMUST)

Tel: 056-30870
Fax: 056-30153
E-mail: directordps@mmust.ac.ke
Website: www.mmust.ac.ke

P.O Box 190
Kakamega – 50100
Kenya

Directorate of Postgraduate Studies

Ref: MMU/COR: 509099

20th February 2023

Kevin Soita
HML/G/01-54970/2020,
P.O. Box 190-50100,
KAKAMEGA.

Dear Mr. Soita,

RE: APPROVAL OF PROPOSAL

I am pleased to inform you that the Directorate of Postgraduate Studies has considered and approved your Masters Proposal entitled: ***"Selected Biomarkers Variability among Type 2 Diabetes Mellitus Patients with Poor Glycemic Control"*** and appointed the following as supervisors:

1. Dr. Nathan Shaviya - MMUST
2. Dr. Godfrey M. Gitonga - MMUST

You are required to submit through your supervisor(s) progress reports every three months to the Director Postgraduate Studies. Such reports should be copied to the following: Chairman, School of Public Health, Biomedical Sciences and Technology Graduate Studies Committee and Chairman, Medical Laboratory Sciences Department. Kindly adhere to research ethics consideration in conducting research

It is the policy and regulations of the University that you observe a deadline of three years from the date of registration to complete your Master's thesis. Do not hesitate to consult this office in case of any problem encountered in the course of your work.

We wish you the best in your research and hope the study will make original contribution to knowledge.

Yours Sincerely,

Prof. Stephen O. Odebero, PhD, FIEEP
DIRECTOR, DIRECTORATE OF POSTGRADUATE STUDIES

Appendix VI: MMUST Ethics Approval

MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY
Tel: 056-31375
Fax: 056-30153
E-mail: ierc@mmust.ac.ke
Website: www.mmust.ac.ke
P. O. Box 190,
50100.
Kakamega,
KENYA

Institutional Scientific and Ethics Review Committee (ISERC)

REF: MMU/COR: 403012 Vol 6 (01) Date: April 14th, 2023

To: Mr. Kevin Soita.

Dear Mr.,

RE: SELECTED BIOMARKERS VARIABILITY AMONG TYPE 2 DIABETES MELLITUS PATIENTS WITH POOR GLYCEMIC CONTROL.

This is to inform you that the ***Masinde Muliro University of Science and Technology Institutional Scientific and Ethics Review Committee (MMUST-ISERC)*** has reviewed and approved your above research proposal. Your application approval number is **MMUST/IERC/143/2023.** The approval covers for the period ***March 14th, 2023 to March 14th, 2024.***

This approval is subject to compliance with the following requirements;

i. Only approved documents including informed consents, study instruments, MTA will be used.
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by ***MMUST-ISERC.***
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to ***MMUST-ISERC*** within 72 hours of notification
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to ***MMUST-ISERC*** within 72 hours
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to ***MMUST-ISERC***.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://research-portal.nacosti.go.ke and also obtain other clearances needed

Yours Sincerely,

Prof. Gordon Nguka (PhD)
Chairperson, Institutional Scientific and Ethics Review Committee

Copy to:
- The Secretary, National Bio-Ethics Committee
- Vice Chancellor
- DVC (PR&I)

Appendix VII: NACOSTI Research permit

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **164250**

Date of Issue: **29/April/2023**

RESEARCH LICENSE



This is to Certify that Mr.. KEVIN SOITA of Masinde Muliro University of Science and Technology, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Kakamega on the topic: SELECTED BIOMARKERS VARIABILITY AMONG TYPE 2 DIABETES MELLITUS PATIENTS WITH POOR GLYCEMIC CONTROL for the period ending : 29/April/2024.

License No: **NACOSTI/P/23/25518**

164250

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

See overleaf for conditions

Appendix VIII: Kakamega County data collection approval

COUNTY GOVERNMENT OF KAKAMEGA

E-mail: wpgh15@yahoo.com
Telephone: Kakamega 0702930346
When replying, please quote:

REF: CGH/KAK/ERC/VOL.I/209

COUNTY GENERAL HOSPITAL
P.O. Box 15-G.P. O-50100
KAKAMEGA

DATE: 16th May, 2023

MINISTRY OF HEALTH SERVICES

KEVIN SOITA,
REF: NACOSTI/P/23/25518

RE: RESEARCH AUTHORIZATION FOR DATA COLLECTION – NO. ERC/200-05/2023

This is to inform you that Kakamega County General Hospital Ethics Review Committee (KCGH ERC) acting on behalf of the Kakamega County Department of Health, has reviewed and authorized your data collection for the protocol titled: ***"Selected Biomarkers Variability Among Type 2 Diabetes Mellitus Patients with Poor Glycemic Control."*** The approval period shall expire on 29th April 2024.

This authorization is subject to compliance with the following requirements:

i. Only approved documents including informed consent, study instruments, will be used.
ii. All changes including amendments, deviations and violations are submitted for review and approval by the **KCGH ERC.**
iii. Death and life-threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to **KCGH ERC** within 24 hours of notification.
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety of welfare of the study participants and others or affect the integrity of the research must be reported to **KCGH ERC** within 24 hours.
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to **KCGH ERC**.
viii. Submission of quarterly progress report to the **KCGH ERC** and dissemination of preliminary findings at the end of the study is expected from the researcher

This authorization should be attached to your research license from National Commission for Science, Technology and Innovation (NACOSTI) and also other necessary clearances. Preliminary dissemination of your study findings to **KCGH ERC** is mandatory prior to publications.

COUNTY DIRECTOR HEALTH SERVICES
17 MAY 2023
Sign.....
P.O. Box 359-50100, KAKAMEGA COUNTY

DR. AJEVI AUSTINE
CHAIRMAN; ETHICS AND RESEARCH COMMITTEE
CGH – KAKAMEGA
Copy to: Director, Health Services

Appendix IX: Map of Kakamega county

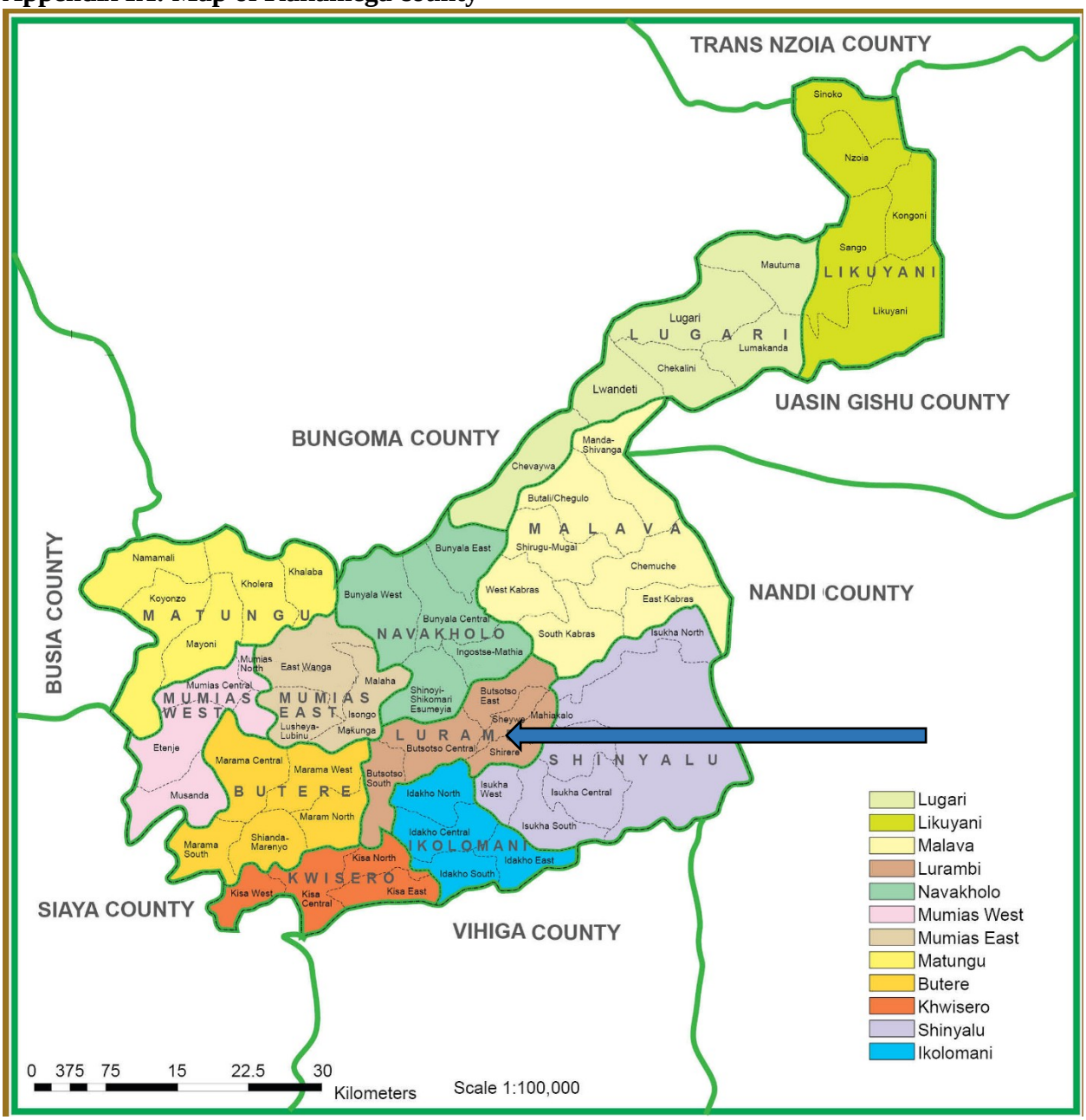

Figure 1.2: *A map of Kakamega county showing various sub counties*