UTILIZATION OF GLYCOSYLATED HAEMOGLOBIN AND MICROALBUMIN IN THE MANAGEMENT OF UNCONTROLLED DIABETES MELLITUS PATIENTS ATTENDING KIBAGABAGA DISTRICT HOSPITAL RWANDA

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Utilization of Glycosylated Haemoglobin and Microalbumin in the Management of Uncontrolled Diabetes Mellitus Patients Attending Kibagabaga District Hospital Rwanda

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

Signature……………………………………………………. Date ………. ………….

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This thesis has been submitted for examination with our approval as the University Supervisors.

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To Eugenia, Yanis Armel, and Aella

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ABBREVIATIONS AND ACRONYMS

AER   Albumin Excretion Rate

ACEi   Angiotensin Converting Enzym inhibitors

ACR   Albumin Creatinine ratio

ARB   Angiotensin Receptor Blockers

AGEs   Advanced Glycosylation End products

CKD   Chronic Kidney Disease

CVD   Cardiovascular Disease

DKA   Diabetes Ketoacidosis

DN   Diabetes Nephropathy

EDTA   Ethylene Diaminetetraacetic Acid

ESRD   End-Stage Renal Disease

ESKD   End-Stage Kedney Disease

ESRF   -Stage Renal Failure

FBS   Fasting Blood Sugar

GDM   Gestational Diabetes Mellitus

GFR   Glomerular Filtration Rate

HBA1C   Haemoglobin A1c

HHNK   Hyperosmolar Hyperglycemia Non-Kenotic

HIV/AIDS	Human Immunodeficiency virus/acquired immunodeficiency syndrome
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KDIGO	Kidney Disease Improving Global Outcomes
LJ	Levey-Jennings
RAAS	Renin-Angiotensin-Aldosterone System
MLS	Medical Laboratory Sciences
MODY	Maturity-Onset Diabetes of the Young
NCD	Non-Communicable Diseases
OGTT	Oral Glucose Tolerance Test
OPD	Outpatient Department
QA	Quality Assurance
QC	Quality Control
UAE	Urinary Abumin Excretion
WHO	World Health Organization
FMD	Fbromuscular dysplasia

ABSTRACT

Diabetes mellitus is a clinical syndrome described by hyperglycemia because of the total or relative lack of insulin. Diabetes has many complications. One such major chronic complication of poorly controlled diabetes is diabetic nephropathy which may prompt end-stage renal disease. The glycosylated haemoglobin (HbA1c) is tested primarily to determine the three-month average blood sugar levels. This helps clinicians to get an overall picture of what the average blood sugar levels have been over a period of months. Long-term control of diabetes is monitored by the estimation of glycosylated haemoglobin (HbA1c). This study recommends the microalbuminuria screening test is recommended annually to assess early renal damage in people who are at risk of developing kidney complications. Microalbuminuria screening test is justified on its benefit in diabetic patients monitoring while the process is still reversible. Microalbuminuria is not yet implemented at Kibagabaga District level due to cost restriction. Furthermore, early onset of renal impairment among diabetes mellitus is recommended using microalbuminuria. The fasting blood sugar is only used routinely to monitor diabetic patient at district levels. The fasting blood sugar is not accurate compared with the glycosylated haemoglobin. However, the macro albuminuria is far costly to glycosylated haemoglobin in term of reagents, materials and consumables. The main objective of this research was to estash a correlation between HBA1c and microalbumin in the management of uncontrolled c diabetes mellitus patients attending Kibagabaga District Hospital.This can be used instead of microalbuminuria at certain level of glycosylated haemoglobin to predict the renal impairment. A hospital-based cross-sectional design was used. The known uncontrolled diabetes mellitus patients were referred by medical team and recruited in the study till the sample size reached with 246 participants from April to June 2018. After sample collection instruction, random urine was collected and microalbuminuria was tested using method of albumin creatinine ratio (ACR). Microalbuminuria was defined as urinary albumin-to-creatinine ratio > 3 mg/mmol, normoalbuminuric as < 3 mg/mmol, and macro albuminuria as >300 mg/mmol. The veinous blood samples were collected in ethylenediaminetetraacetic acid (EDTA) tubes and Sodium Fluoride for HBA1c and FBS measurement respectively. Uncontrolled diabetes mellitus was defined as a cut-off value of HBA1c > 6.5 %. Data ware analyzed by statistical software SPSS version 21. Pearson correlation coefficient and Chi square were calculated to find the relationship between variables. P value was taken as significant at 5 percent confidence level (P<0.05). Out of 246 patients, 178 (72.36%) were female and 68 (27.64%) were male. The age range was 31 to 81 years old and the average age was 58 years old. The duration of diabetes range was 1 to 19 years with an average of 7 years. Eighty-one (32.9%) of study subjects had microalbuminuria and one hundred sixty-six (67.1%) had normoalbuminuric and no cases of macro albuminuria were recorded. The correlation between Microalbuminuria and HBA1c was significantly positive (r=0.800 and p < 0.05) and duration of diabetes (0.664 and p < 0.05). This correlation between microalbuminuria and HBA1c results among uncontrolled diabetes patients helped us to propose the access to glycosylated haemoglobin (HbA1c) testing at the district hospital level. This will also assist to predict the kidney complication among uncontrolled diabetes patients with HBA1c above 14%. This study recommends that HBA1c should be inculcated in routine practice for uncontrolled diabetes mellitus patients and Microalbumin test should be checked at a

regular interval: if detected microalbuminuria is detected, confirmation should be made with two further tests within a 3-to-6-month period. If microalbuminuria is not detected, re-screening should be performed annually. This study recommends the ministry of Health to implement a guide line for monitoring the uncontrolled diabetes mellitus and the early diabetes nephropathy diagnosis at the district hospitals.

CHAPTER ONE

INTRODUCTION

1.1. Background information

Diabetes mellitus is a heterogeneous arrangement of metabolic impairment represented by hyperglycemia (Fac *et al,* 2005). In 2011 the estimated number of diabetic cases globally was 366 million with the projection for 2030 standing at 552 million individuals. The biggest increment (92%) in cases has been attributed to countries in the lowest income group (Babu *et al.,*2013). (WHO, 2016) reported 1.5 million deaths diabetes alone and 2.2 million deaths due to diabetes complications in 2012. Forty-three percent of these 3.7 million deaths happen before the age of 70 years. Etiological classification of disorders of glycaemia has helped to distinguish Type 1 (insulin deficiency) into autoimmune and idiopathic, Type 2 (insulin resistance), other specific types also known as type 3 diabetes (genetic beta-cell defects, pancreatitis, etc.), and Gestational diabetes (type 4 diabetes mellitus) (Alberti & Zimmet,1998). The classical group of three diabetes symptoms is polyuria, polydipsia, and polyphagia which are frequent urination expanded thirst, and expanded appetite (Zhang *et al.*, 2015).

Hyperglycemia is a typical impact of uncontrolled diabetes mellitus is related with the development of various inconveniences, the fundamental ones being nephropathy, neuropathy, retinopathy, and cardiovascular diseases (Kinmonth *et al.,* 2010).

Diabetic nephropathy influences around 15 to 25% of type 1 diabetic patients and 30 to 40% of patients with type 2 diabetes (Schrijvers *et al.,*2004). Cohort studies have shown that the diabetic life expectancy of type 1 diabetic patients is around 10 years after proteinuria diagnosis is made, with 66% of deaths related end-stage renal failure (ESRF) and 34 % to cardiovascular diseases. In type 2 diabetes, because of the nearness comorbid conditions and cardiovascular diseases in the overt nephropathy, the mortality chance is phenomenal to the point that many passes on before reaching End-Stage Renal Failure (ESRF)(Gosmanov, Wall, & Gosmanova, 2014).The correct

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reason of diabetic nephropathy is obscured however the expanding proof demonstrates that hyperglycemia is the starting reason for the tissue damage initiates diabetes, either through rehashed intense changes in cell glucose metabolism or through the long-term amassing of glycosylated biomolecules and Advanced Glycosylation End-products (AGEs) (Negre *et al.,* 2009).Two imperative foci for the subsequent care of people with diabetes incorporate checking of glycemic status by estimation of glycosylated haemoglobin (HbA1C) and screening for kidney microalbuminuria to evaluate kidney disease and to recognize potential progression toward end-organ risk (Golden *et al*., 2003). Subsequently, one of the significant objectives of treatment of diabetes mellitus is to keep blood glucose levels as near the normal as would be prudent yet avoiding hypoglycemia by keeping up glycosylated haemoglobin(HbA1c) beneath a specific level, along these levels limiting the risk of complications of diabetes mellitus (Babui *et al*., 2013).Glycosylated haemoglobin measurement is a method for estimating the degree of glycaemia for 2 to 3 months. Diabetic nephropathy is probably to develop in patients with high HbA1c control(Muraliswaran *et al.,*2016). Screening for microalbuminuria among people with diabetes is additionally suggested for the diagnosis and treatment prescription to slow the progression of kidney disease(Golden *et al*., 2003). The high glycosylated haemoglobin (HbA1c) is identified to induce microvascular diseases in DM, and its diminishment is at the focal point of the clinical management of hyperglycemia (Bash *et al.,* 2008). Microalbuminuria is a key element of diabetic nephropathy, a solid indicator of progression towards ESRD. Normal microalbuminuria (albumin:30-300mg/day or albumin excretion: 20–200 (μg/min) or Albumin-to-creatinine ratio:3.0–35(mg/mmol) is described as microalbuminuria and the patient with microalbuminuria are alluded to have incipient nephropathy (Muraliswaran *et al*.,2016). The glycosylated haemoglobin (HbA1c) test is normally the most utilized measure of long-term glycemic control. HbA1c levels in diabetes for a normal control will be < 6.5% and that of 7.5% will be at greater risk. Most of the randomized clinical trials have shown that tight glycemic control stops the development and progression of long-term diabetic microvascular complications (Golden *et al.,*2003).

1.2. Statement of the Problem

The diabetes prevalence is growing in every country, 4 out of 5 diabetic cases live in low and middle-income countries and half of the diabetics don't know they experience its complications (Bregu *et al.,*2012). Noubiap *et al*., 2015 (a) have reported the greatest increment of diabetes prevalence will occur in sub-Saharan Africa, with a projection of 109.6%, from 19.8 million in 2013 to 41.5 million in 2035. In 2013, only 8.6% of all deaths in sub-Saharan Africa were caused by diabetes, 76.4% of these deaths happened in individuals younger than 70 years, contrasted with the half or less among diabetes-related deaths around the world (Tapela *et al*., 2016).Diabetes can prompt complications in numerous organs of the body and increase the high risk of dying prematurely. Probable complications include heart attack, stroke, kidney failure, leg amputation, vision loss and nerve damage (WHO 2016). It has been evaluated that over 40% of individuals with diabetes will develop chronic kidney disease (CKD), including an important number who will develop ESKD requiring renal replacement treatments (dialysis or transplantation) (Bregu *et a*l., 2012). In Rwanda, the prevalence of DM was evaluated to be 3.06% (WHO, report 2016). The national prevalence of diabetic nephropathy is not known. However, the hospital-based study conducted by Rudasingwa *et al.,*2012   on diabetes complications have found diabetes mellitus microvascular neuropathy (53%), retinopathy (23%), and nephropathy (20%), and macro vascular complications (4%) diagnosed based on clinical findings. The diabetic retinopathy and neuropathy are screened based on clinical exams (on reflexes and sensation, eyes check, blood pressure) while the microalbuminuria test is recommended for diabetic nephropathy to detect the beginnings of kidney damage very early and use medications to prevent any further damage (Gupta. 2015).

Jamison *et al.* 2006 have confirmed that very many countries in Sub Sahara cannot afford screening and treatment for DM complications. Both studies have demonstrated that in most African countries, HbA1C and microalbuminuria tests were not done as recommended for long-term glucose monitoring and early diagnosis of diabetic nephropathy because of lack of laboratory facilities. At Kibagabaga District Hospital, the diabetics are diagnosed and followed up based on fasting blood sugar (FBS) results

only. This study will cover the laboratory test gap for uncontrolled diabetes mellitus monitoring to evaluate the treatment efficacy and early kidney complication diagnosis at Kibagabaga District Hospital; where a whole blood and urine samples will be collected and send to the laboratory of University Teaching Hospital of Kigali laboratory (UTHK) for HbA1c and microalbuminuria testing. The obtained results will help to establish the correlation between HbA1C and microalbuminuria in the management of uncontrolled diabetic patients attending Kibagabaga District Hospital.

1.3. Justification

Diabetic nephropathy is a kidney disease that results from diabetes complications. Diabetes nephropathy is the main source of chronic kidney failure worldwide and is responsible for kidney failure in around 33% of patients on dialysis treatment (Dabla, P. K. (2010). Screening for diabetic nephropathy must be begun after diagnosis of type 2 diabetes since 7% of them have microalbuminuria. For patients with type 1 diabetes mellitus, the first screening has been proposed at 5 years after diabetes confirmation. However, the prevalence of microalbuminuria before 5 years in type 1 diabetes mellitus can achieve 18%, especially in uncontrolled diabetes patients (Gross *et al*., 2005). Timely and accurate glycemic control may prevent the anticipation or delay diabetic nephropathy. The regular glycemic control likewise lessens the occurrence of micro and macro albuminuria by 39% and 54%, respectively (Dounousi *et al.*.2015). Measurement of glycosylated haemoglobin has turned into the best quality level strategy for surveying long-term glycemic control. HbA1c is the favored standard for measuring glycemic control over the past 2-3 months (Golden *et al*.,2003).

The fasting blood sugar (FBS) has been appeared to be inconsistent because of the many elements which are known to influence glucose levels in the blood (ie. age, time of day, push, suppers, and so forth.). Also, it has been observed that diabetic patients may fleetingly enhance their consistency preceding facility visits and in this way information in light of center checking may yield one-sided appraisals of their level of glucose control (WHO, 2002). The results obtained from this study will help to establish a correlation between HbA1c and microalbuminuria in the management of uncontrolled diabetes at Kibagabaga District Hospital. The correlation will predict the

early kidney damage where a health facility is unable to perform the microalbuminuria test in particular.

1.4. Research Questions

This was tackled through three specific research questions:

1) What was the level of glycosylated haemoglobin in uncontrolled diabetic patients attending Kibagabaga hospital?
2) What was the level of microalbuminuria in uncontrolled diabetic patients attending Kibagabaga hospital?
3) Was there a correlation between glycosylate haemoglobin and microalbumin among study subjects?

1.5. Hypothesis

There was no correlation between glycosylated haemoglobin and microalbumin (null hypothesis) in the management of uncontrolled diabetes mellitus patients attending Kibagabaga District Hospital.

1.6. Objectives
1.6.1. General objective

To establish a correlation between glycosylated haemoglobin and microalbumin in the management of uncontrolled c diabetes mellitus patients attending Kibagabaga District Hospital.

1.6.2. Specific Objectives

1) To determine the glycosylated haemoglobin (HbA1c) levels in the uncontrolled diabetes mellitus patients attending Kibagabaga District Hospital
2) To determine the microalbunia concentration in the uncontrolled diabetes mellitus patients attending Kibagabaga District Hospital

3) To establish the correlation between HbA1c and microalbuminuria, FBS, Ages, Gender and diabetes duration among the study subjects.

CHAPTER TWO

LITERATURE REVIEW

2.1. Diabetes mellitus

Diabetes mellitus is a complex, incessant sickness. It is a condition depicted by an abnormal state of glucose in the blood. Insulin, a hormone made by the pancreas, modulates the blood glucose level by regulating the production, storage, and secretion of glucose in the peripheral circulation. In diabetes, there may be a diminished ability in the body to respond to insulin or a reduction in the insulin conveyed by the pancreas. These changes prompt anomalies in the metabolism of carbohydrates, proteins, and fats. The resulting hyperglycemia may incite extreme metabolic consequences incorporating ketoacidosis in the short-term and eventually progressing to microvascular complications with time (Gupta *et al*., 2015).

2.1.1. Signs and symptoms of diabetes mellitus

The signs and symptoms of diabetes of mellitus occur as a result of a high blood sugar level in the blood. There is polyuria (increase urine output) which comes about because of glycosuria and polydipsia (expanded thirst). Polydipsia is auxiliary to osmotic dieresis and hyper osmolality while polyphagia (increased hunger) comes about following cell starvation, and diminished stockpiling of calories. Weight reduction on the foundation of polyphagia is because of the insufficient digestion of sugar, protein, and fat. Weakness and lethargy are experienced as a result of inadequate energy production. Weakness is another side effect of diabetes mellitus. Wounds require a long duration to mend because of the lacking blood supply to the lower limits. Growth disturbances, immune deficiencies and infections (such as vaginitis) in particular, may accompany hyperglycemia. The diabetics may also experience obscured vision (Zhang *et al.,* 2015). The hypoglycemia (below normal blood sugar) is most usually observed as symptoms of insulin treatment or with the utilization of oral hypoglycemic treatments in patients with diabetes mellitus (Bregu *et al.,* 2012).

2.1.2. Classification of diabetes mellitus

Diabetes is classified into four general types: Type 1 diabetes, formerly called juvenile-onset or insulin dependent diabetes (due to β-cell destruction) typically prompts a total lack of insulin. Type 2 diabetes (due to a progressive insulin secretory defect) is the foundation of insulin resistance. Type 3 also known as gestational diabetes mellitus (GDM) is diabetes analyzed in the second or third trimester of pregnancy that is not overt diabetes. Type 4 also known as specific types of diabetes because of different causes, e.g., monogenic diabetes disorders, (such as neonatal diabetes and maturity-onset diabetes of the young [MODY]), illnesses of the exocrine pancreas, (for example, cystic fibrosis), and medications or chemical-induced diabetes, (for example, in the treatment of HIV/AIDS or after organ transplantation (Zhang *et al..,* 2015).

2.1.3. Diagnostic criteria for diabetes mellitus

As per the reconsidered criteria by the expert committee of diabetes in collaboration with WHO, came up with the new limits for the fasting plasma glucose (FPS) ≥125 mg/dl (7.0mmol/l), 2-hours plasma glucose ≥ 200 mg/dl (11.1mmol/l) during OGTT. The increased risks or pre-diabetes values were updated to FPG 100-125 mg/dl(5.5-6.9 mmol/l) for IGT and 2-hours plasma in 75 mg OGTT 140-199 mg/dl(7.8-11.0 mmol/l) for IGT (Straseski, 2013).

2.1.4. Diabetes mellitus complications

The complications of diabetes mellitus might be isolated into two main classifications: acute and chronic. The acute complications are specifically identified with the fast change in digestion and incorporate diabetes ketoacidosis (DKA), hyperosmolar hyperglycemia non kenotic coma (HHNK), and hypoglycemia. These complications are, to some degree, preventable and require prompt remedial interventions (Bash *et al.,* 2008). The complications occur because of the chronic exposure of the body's tissues to hyperglycemia: microvascular (diabetic retinopathy, diabetic neuropathy, diabetic nephropathy, and diabetic skin problems and macro vascular (atheroma,

coronary hearPresst disease, myocardial infarction, arteriosclerosis, hypertension, and cerebrovascular disease) (Press,Dove, 2014)

2.2. Diabetes nephropathy

Diabetic nephropathy (DN) or diabetic kidney is a disorder portrayed by a dynamic increment in the urinary proteins excretion, particularly an early urine albumin proceeding with ascending in systematic blood pressure (BP), and late decrease in glomerular filtration rate (GFR), driving inevitably to end-stage renal disease (ESRD) (Salah *et al.,* 2002).

2.2.1. Epidemiology of diabetic nephropathy

As of now, diabetic nephropathy is the main source of unending kidney malady (CKD) in the United States and other Western Societies. Diabetes is in charge of 30-40% of all ESRD cases in the United States. The estimated overall incidence rate of CKD and end-stage renal disease (ESRD) in India is currently 800 per million populations and 150-200 pmp, respectively. It has observed that DM as the cause of CKD found in 31.2% of patients (Gosmanov *et al.,* 2014). The main life-threatening complication develops in around 20% to 40% of type 1 and under 20% of type 2 diabetic patients. Diabetic nephropathy is the main known reason for ESRD (Jawa *et al*., 2004)

2.2.2. Natural history of diabetic nephropathy and screening

It is perceived as a complication of diabetes as a result of changes in the kidney auxiliary to hyperglycemia. In diabetic patients, this comprises of a urinary albumin excretion of 30–200 mg/24 hours, since rates inside this range have been appeared to foresee the progression of diabetic nephropathy (Jawa *et al*., 2004). The earliest clinical proof of diabetes nephropathy (DN) is microalbuminuria characterized as microalbumin in a spot urine collection. A more prominent extent of patients with type 2 diabetes compared with type 1 diabetes have microalbumin and overt nephropathy at or soon after the diagnosis of diabetes (Ayodele *et al.,* 2004).

2.2.3. Pathophysiology of diabetes nephropathy

The pathophysiology of diabetic nephropathy isn't completely caught on. DN is caused by both metabolic alteration and hemodynamic modification. Different components, for example, inflammation, endothelial alteration and oxidative anxiety are likewise under scrutiny. Oxidative anxiety devours nitric oxide, which anticipates flow-mediated dilation of veins, subjecting the endothelium to damage. This prompts the creation of cytokines, increasing the speed of aggravation, compounding of vein inflexibility because of atherosclerosis, and further hindrance of fibromuscular dysplasia (FMD) and powerlessness to oxidative anxiety. Aggravation, endothelial alteration, and oxidative anxiety can be thought of as an "endless loop" that prompts noteworthy kidney damage and cardiovascular disease. A key part of the pathophysiology is basement cellar layer damage. With renal damage, there is dynamic thickening of the basement cellar layer, obsessive change in mesangial and vascular cells, development of Advanced Glycation End-products (AGEs), aggregation of polyols through the aldose reductase pathway, and enactment of protein kinase C. Entry of macromolecules through the storm cellar layer may likewise enact incendiary pathways that add to the damage optionally(Bennett & Aditya, 2015).

2.2.4. Microalbumin and diabetic nephropathy

Microalbuminuria is an early part in a continuum of dynamic progressive in albumin excretion rates AERs) that normally describes DKD. The term alludes to a subclinical increase in AER. By definition, it relates to an AER of 20–200 mg/min (30–300 mg per day) or an albumin: creatinine ratio of 2.5–35 mg/mmol in male subjects and 3.5–35 mg/mmol in female subjects. Therefore, microalbuminuria alludes to modest quantities and not the size of urinary albumin or albumin-derived particles (Keri *et al.,* 2018). The terms 'normoalbuminuric', 'microalbumin' (nascent DN) and 'macro albuminuria' don't allude to discrete, subjectively unique parts of DN. Note that the lower furthest reaches of microalbumin surpasses the maximum furthest reaches of AER in typical subjects, and late investigations have demonstrated that AER levels in the scope of 10– 20 μg/min additionally anticipate progression to overt DN (Geoffrey Boner and Mark Coopper 2003).

2.2.5. Clinical stages of diabetic nephropathy

The clinical stages are arranged on the premise of the estimations of GFR, urinary albumin excretion (UAE), and arterial blood pressure. Also because of increment cardiovascular mortality, many types 2 diabetic patients pass on before they develop four and five (Gosmanov *et al.,* 2014). Stage 1 or initial nephropathy is characterized by, the kidney increments in size accompanied by high filtration and priming rate. Patients need to entirely control their glucose at this stage. Quiescent or stage 2 is settled by five years after the onset of the diabetes mellitus and is exemplified by thickening of the glomerular cellar layer, mesangial extension, and nephromegaly additionally been suggested as essential pathogenetic systems (Nand *et al.,*2001). Glycosylation of the cellar layer has been appeared to happen and may bring about the expanded filtration of proteins. The structure of the kidney is changed for more terrible and patients pass protein in their urine after intense physical movement. They are required to rest however the rest is recommended at this stage (Gosmanov *et al..* 2014).Stage 3 or incipient nephropathy is generally appeared 10-15 years after the term of hyperglycemia and is marked by the microalbuminuria (30 mg/24 hours or 20 ug/min), hypertension and further mesangial changes (Williams *et al.,* 2002). Microalbuminuria is characterized by a day-by-day ACR deterioration and a poor glomerular filtration output. It is additionally associated with a vascular complication in different organs. Around 30 to 40 percent of patients would have an advanced state of proteinuria at this stage and their renal capacities start to decrease (Gosmanov *et al.,* 2014). Stage 4 or overt nephropathy is described by expanded proteinuria, hypertension and progressive glomerular filtration rate are also present (Gosmanov *et al.,*2014). The event of macro albuminuria peaks in patients who have had diabetes for 15 to 20 years. Without mediation, the GFR in macroalbuminuric patients with type 1 diabetes falls at around 1 mL/min/mo. The nephrotic disorder is likewise extremely frequent(Mogensen *et al..*1983).

Jawa *et al.*, 2004 have described stage 5 or End-stage renal disease or uremia where the patient's condition is worse. They have to experience dialysis and kidney transplant to manage their life. The patients are constantly stressed over their condition in this phase of the ailment. The choice to take dialysis or not is continually confronting them

even though the procedure just disposes of poisons in the body. Mogensen *et al*., 1983 have prescribed for such patients to take other treatments and not rely upon dialysis to get a break from side effects. Also because of increment cardiovascular mortality, many types 2 diabetic patients pass on before they develop four and five stages.

2.2.6. Risk factors for diabetic nephropathy

There are several major risk factors for the development of diabetic nephropathy with the most important of these probably being the duration of diabetes. The risk of developing ESRD also varies with ethnic origin. The age and gender-adjusted the rate of ESRD stratified by ethnicity and the primary diagnosis. Other major clinical determinants of diabetic nephropathy are hypertension, hyperglycemia, smoking, dyslipidemia, and genetic factors (Tandon *et al.,* 2015)

2.3. Glycosylation of proteins

Proteins respond precipitously in the blood with glucose to shape glycosylated subordinates. This response happens gradually under physiological conditions and without the association of catalysts. The degree of glycosylation of proteins is controlled by the convergence of glucose in the blood and by the quantity of responsive amino gatherings show in the protein that is available to glucose for a response. All proteins with receptive destinations can be glycosylated and the convergence of the glycosylated proteins that can be measured in blood is a marker for the vacillation of blood glucose focuses amid a specific period. From a clinical indicative point, glycosylated proteins with a more drawn out life in the blood are of interest since they mirror the presentation of these proteins to glucose for longer periods (WHO, 2002)

2.3.1. Biosynthesis and biology of glycosylated haemoglobin

HbAlc is formed in vivo as the result of a non-enzymatic post-translational modification in which the amount of end-product formed is dependent upon the average glucose concentration over the 120-day lifespan of the erythrocyte. The reaction is a two-step process in which glucose initially combines with the N-terminal

valine of a P-chain to form an unstable aldimine which then undergoes an Amadori rearrangement to form a stable ketoamine (WHO, 2002).

2.3.2. Advanced glycosylation end products and diabetes nephropathy

Advanced glycosylation end products (AGEs) are a heterogeneous gathering of protein and lipids to which sugar deposits are covalently bound. AGE arrangement is expanded in circumstances with hyperglycemia (e.g., diabetes mellitus) and is additionally fortified by oxidative stress, for instance in uremia. It creates the impression that the enactment of the renin-angiotensin framework may add to AGE arrangement through different systems. In spite of the fact that AGEs could nonspecifically tie to the basement membrane and change their properties, they additionally initiate particular cell reactions including the release of profibrogenic and proinflammatory cytokines by collaborating with the receptor for AGE (RAGE). The AGEs are associated with the basic changes of dynamic nephropathies, for example, glomerulosclerosis, interstitial fibrosis, and tubular decay. These impacts are most conspicuous in diabetic nephropathy(Bohlender *et a*l., 2005).

2.4. Prognosis of diabetic nephropathy

The prognosis for those with diabetic nephropathy is variable and depends on the individual's factors, the type of diabetes, and the complications of finding and the treatment. The phase of microalbumin, as a rule, advances more than 10– 15 years with characterized initiators and promoters. After the change to the microalbuminuric or the overt nephropathy stage, the rate of progression renal illness is impacted by various variables including the level of blood pressure, hyperglycemia, and level of proteinuria or albuminuria, the nearness of retinopathy, smoking and potential frailty(Tobergte, D. R., & Curtis, S. (2013).Once incipient diabetic nephropathy changes into overt nephropathy, progression can't be ceased. Hence, it is more imperative to screen the patients for early nephropathy(Gupta & Singh, 2017). In Type 1 diabetes patients with clinical diabetic nephropathy, end-renal stage /uremia is the commanding reason for death, in charge of about 60% of all deahts. In Type 2 diabetes patients the relationship among proteinuria and cardiovascular disease is significantly

more grounded, with the vast majority of the patients dying from CVD before consistent end-stage renal failure progression (Donnelly, 2005).

2.5. Screening and diagnosis of diabetic nephropathy

Urine analysis and the right account of the history of supine or erect blood pressure must be done. Patients ought to be screened for microalbuminuria in the diabetic center. Microalbuminuria is measured as the most punctual clinically distinguishable confirmation of DN. It is imperative to take note of the transient increment in urinary albumin excretion (UAE) that can be caused by uncontrolled hyperglycemia; or hypertension, fever, urinary tract disease, congestive heart disappointment or physical effort. In this way, it is proposed that microalbuminuria ought to be affirmed by a repeating microalbuminuria test over the accompanying 3-6 months. The results are interpreted using KDIGO 2012 CKD guidelines as follows; normal to mildly increased (< 3mg/mmol), moderately increased: microalbuminuria (3-30 mg/mmol) and severely increased (>30 mg/mmol. The albumin/glycaemia ratio (ACR) can be performed in the early morning or random urine spot. An ACR of 2.5 is normally taken as the cut-off for microalbuminuria (proportionate to a UAER of >30 mg/24h). Once a patient develops proteinuria, it is important to rule out causes other than diabetes. This is particularly important in type 1 diabetes. In these cases, patients must be evaluated for other conditions such as hepatitis B and C, human immunodeficiency virus, lupus nephritis, and myeloma, as well as the use of the non-steroidal anti-inflammatory drug (Gosmanov *et a*l. ,2014).

2.6. Management of diabetic nephropathy

Treatment of DN ought to be tended to given the clinical phase of the disease process. DN can be averted if analyzed and overseen ahead of schedule by utilizing basic counteractive action techniques, including firm control of glucose and blood pressure monitoring. Treatment of dyslipidemia by lifestyle enhancement including those relating to diet, physical excise and weight decrease is viewed as a commendable essential mediation for DN patients. Smoking discontinuance can essentially decrease cardiovascular risk and should be supported. There is confirm that smoking end

enhances the progression of microalbuminuria to macro albuminuria and enhances renal anticipation(Gosmanov *et a*l., 2014).

2.6.1. Glycemic control

Glycemic control is fundamental to delay the beginning of diabetes complications, and it can challenge even the most experienced doctor. Glucose control in those with CKD includes another level of intricacy. It requires itemized learning of which prescriptions can be securely utilized and how kidney illness influences the digestion of these solutions(Hahr & Molitch, 2015). Glycosylated haemoglobin (HbA1c) levels help to diagnose both pre-diabetes 5.7 to 6.4% and diabetes (above 6.4%). While it is uniformly accepted that higher HbA1c levels are associated with greater risk of complications from diabetes. In March of 2018, the American College of Physicians recommended to de-intensify pharmacologic therapy when HbA1c < 6.5 %(Qaseem *et al*.,2018)

2.6.2. Control of blood pressure at 120/70 mmHg

Enhanced circulatory strain control in diabetic nephropathy regularly requires different antihypertensive medications. Beginning with an ACEi or ARB consolidates a proof-based technique tending to both blood pressure bringing down and albuminuria by focusing on the impacts of Ang II (Nand *et al*., 2001) . Utilizing either loop or thiazide diuretics addresses volume affectability as well as limits the risk for hyperkalemia with renin-angiotensin-aldosterone system (RAAS) blocking medications and upgrades their antiproteinuric impacts. Adherence to a low sodium diet routine is likewise suggested given this guideline (Peter *et al.* 2011).

2.6.3. Early detection and management of diabetes

Having a first-degree relative with DM is a solid risk factor. In ladies, GDM builds the odds of creating T2DM by seven-fold.10 40% of ladies who create GDM in pregnancy will create DM inside 5 years, particularly with expanding age. Early identification of

persons at risk for kidney disease provides an opportunity to prevent or delay its progression and decrease morbidity and mortality (Ofori & Unachukwu, 2014).

2.6.4. Diet and diabetic nephropathy

The part of the dietary protein in the development and progression of diabetic nephropathy is debated while it is characterized that a moderately low protein eating regimen is the best approach for treating the renal illness of diabetic patients (Pedrini *et al*., 1996). Dietary protein confinement moderates the prognosis of renal sickness and enhances survival in creatures with differs glomerulopathies. As of late, a meta-proposed that dietary protein confinement brings down the occurrence of end-stage renal disease (ESRD) or demise in patients with non-diabetic nephropathies and moderates the progression of diabetic nephropathy(Van Buren & Toto, 2011).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study site

This study was conducted in Internal medicine out patient's department of Kibagabaga District Hospital. It is located in Kigali city, in Gasabo District, in Kimironko sector. Kibagabaga is a 230-bed hospital that serves sixteen health clinics in the area. Its specialties include emergency medicine, pediatrics, internal medicine, obstetrics and gynecology, neonatology, ear, nose and throat, mental health, imaging, lab and some general surgery.

3.2. Study design

This study was a hospital-based cross-sectional study.

3.3. Study population

Uncontrolled diabetes mellitus patients attending internal medicine (OPD) at Kibagabaga District Hospital from April to June 2018.

3.4. Inclusion criteria

Known uncontrolled type 2 diabetic cases attending Kibagabaga district hospital with treatment were included in the study.

3.5. Exclusion criteria

Diabetic patients with any of the following characteristics were excluded from the study: Patients who had type 1 diabetes mellitus, chronic kidney disease, smokers, alcoholics, patients on nephrotoxic drugs, patients with primary hypertensive, pregnant female, anemia, haemoglobinopathies disorders associated with accelerated red cell turnover such as malaria medication.

Those patients with uncontrolled type 2 diabetes who declined to give consent to participate were also excluded.

3.6. Sample size determination

Following formula by Bartlett, 2001 was used to determine the minimum sample size:

$N = (t^2 pq)/ d^2$

where:

N=calculated sample size

t=1.96

p=Prevalence rate of uncontrolled diabetes reported by Rudasingwa *et al*. 2012 was 20%

q=1-p

d=error at 95% confidence interval, which is 0.05

Therefore:

$N= \underline{(1.96^2) (0.2) (1-0.2)} = 246$ study subjects

$\qquad 0.05^2$

3.7. Sampling design

The medical team assisted to select all eligible patients through the files before the start of the day's clinic session. Each Uncontrolled diabetic patients who met the inclusion criteria was given full explanation of the study and enrolled after giving written informed consent. Data collection is started by using capturing demographic data of the participants, laboratory investigations and record analysis. The sampling

process came to an end when the sample size of participants and/or the study period was reached.

3.8. Laboratory procedures

3.8.1. Quality Assurance (QA)/ Quality Control (QC)

To ensure accuracy and precision of the test results, all pre analytical, analytical and post analytical precautions were taken into consideration. Internal QC materials from Roche diagnostics; Normal and pathological QCs were run twice a day (morning and evening) during the analytical period according to the manufacturers' instructions and QC protocols. During the entire analytical period, everyday control value results and the standard deviation (SD) from the control target value were noted. All the daily QC runs were plotted on LJ graphs to evaluate the analytical processes. The samples were run when IQCs results were within ±2SD of IQCs charts while when IQC were/was out of ±2SD troubleshooting was conducted and no samples were run till the issue was fixed.

3.8.2. Sample collection

Blood and urine samples from the uncontrolled diabetic patients were the specimen of choice. The sample collection was done during the clinic visit between April to June 2018.

3.8.3. Blood collection procedure

- Wash your hand and gathering phlebotomy materials (vacutainer needle, vacutainer adapter, alcohol pad, tourniquet, cotton ball, tap or band aid, label and pen)
- Introduce yourself and verify the subject's identification
- Wash your hand and wear gloves
- Have the subject sit in a comfortable position

- Palpate a vein and look for the best site for blood draw.

- Clean with alcohol swab in a circular motion stating from the inside going outwards and allow alcohol to dry

- With your non dominant hand stabilize the vein. With your dominant hand, puncture the skin at a 30-degree angle using vacutainer apparatus.

- Obtain blood samples respecting the order of draw EDTA first then Sodium fluoride tube.

- If blood flows freely, the tourniquet may be loosened. Remove the tourniquet just before the last blood sample has been obtained.

- Apply a clean gauze pad over the puncture site and withdraw the needle.

- Apply the needle safety device and discard the needle with the safety device in the sharp's container.

- Invert the collection tubes in additives for the proper mixture.

- Apply pressure to the collection site for approximately 2 to 3 minutes.

- Tap gauze or band aid may be applied.

- Label the collection tubes

- Discard the blood contaminated products

Urine sample collection procedure

A random urine sample was collected by study subjects themselves in the wash room following the instruction given by the investigator:

- Open the urine collection cup, and place on the counte,

- Urinate a small amount into the toilet then stop.

- Continue to urinate into the cup provided.

- stop collection before you finish urinating.

- Finish urinating into the toilet.

- Flush toilet and wash your hands with soap and water.

- Submit the sample to lab as possible.

- Delays may affect some results or result in the sample being rejected beside you procedure in the sterile labeled container for determining the albumin-to-creatinine ratio.

- Once the specimens were acquired, they were labeled with the study numbers and submit to the lab.

3.8.4. Specimen Transportation, Processing, and Storage

Specimens were transported from Kibagabaga District Hospital to the processing laboratory at the University of Rwanda in the College of Medicine and Health Sciences in the laboratory of Biomedical Laboratory Sciences on ice packed cool boxes within 1 hour. Urine samples were aliquot in duplicate vials (2x5ml). All specimens were then stored at 2-8°C awaiting laboratory testing.

3.8.5. Instrumentation for analysis of diabetes

Albumin, creatinine (in urine) and HbA1c (whole blood) was measured by an immunoturbidimetric method using an automatic analyzer (COBAS C111, Roche Diagnostics GmbH, Mannheim, Germany). Microalbumin was calculated using the albumin creatinine ratio (ACR) method of random urine spot.

3.8.6. Reagent preparation for sample analyses

All related reagents are commercially ready for use in the form of cartridges. They are fitted to the required volumes and concentration. Patented reagent carrier design plus

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inbuilt refrigeration prevents reagent evaporation and degradation, ensuring long-term onboard stability and long calibration intervals.

3.8.7. Test calibration

To ensure that the recovered values from the patient samples to be assayed were both accurate and precise, a specific calibration procedure was run according to the Roche diagnostic requirement. Periodic calibration was done according to the manufacture instructions to maintain the accuracy of results.

3.8.8. Processing of patient sample data

The Fasting blood sugar was performed on Sodium Fluoride samples, HBA1c on whole blood (EDTA) while urine albumin and creatinine ration results were used to calculate micro albumin.

3.8.9. Data management and analysis

The raw data were compiled in Microsoft Excel (Ms2007). Statistical analysis was done by applying the Pearson correlation and Chi square test using Statistical Program for Social Sciences (SPSS) version 21 to find the relationship between variables. P value was taken as significant at 5 percent confidence level ($P<0.05$). P-value was taken as significant at a 5 percent confidence level ($P<0.05$). Significance level represented as; (*) less significant ($p<0.05$) (**) significant ($p<0.01$) (***) highly significant ($p<0.001$). Data were presented in the form of tables, graphs, and charts.

3.8.10. Ethical approval

Approval was sought from the Institutional Research and Ethics Committee of the University of Rwanda (college of medicine and health sciences) and permission from the hospital authorities. Participants were fully informed about the purpose, procedures, risks, and benefits of participating in the study. Those who agreed to participate signed the inform consent forms. Data collected were used for the intended

purpose only, kept confidential and stored securely in a lockable cabinet and soft copy saved on the laptop with passwords.

CHAPTER FOUR

RESULTS

Table 4.1: Demographic data of study participants

Gender	Number (n)	Percentages	Age		Diabetes duration
Male	68	27.6	Mean	54.69	4.99
			95% CI	52.61-56.77	4.07-5.90
			Std	8.589	3.767
			Min	38	1
			Max	81	14
			Range	43	13
			IQR	11	4
Female	178	72.4	Mean	57.77	7.08
			95% CI	57.77-60.57	6.33-7.84
			Std	9.468	5.102
			Min	31	1
			Max	79	19
			Range	48	18
			IQR	13	10

The table 4.1 shows that out of 246 patients, 178 (72.36%) were females and 68 (27.64%) were males. Mean ± SD of ages in years for males and females were 54.69 ± 8.589 and 57.77± 9.468, respectively. Mean ± SD of the duration of diabetes in years for males and females was 4.99 ±3.767 and 7.08 ±5.102, respectively.
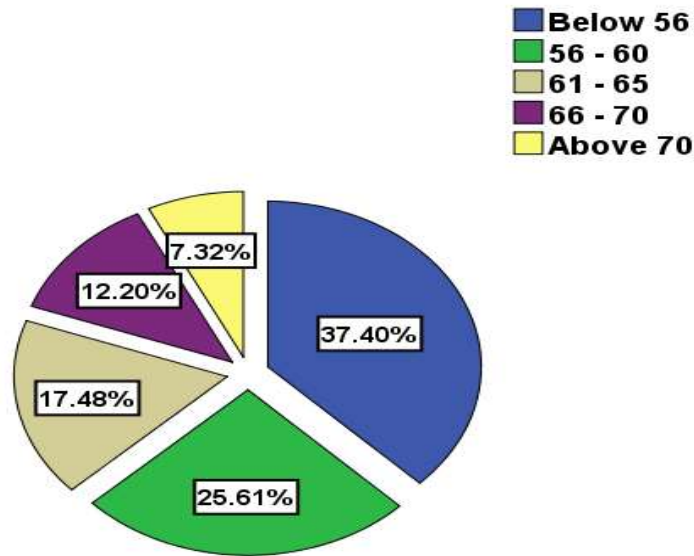
Figure 4.1: Age Categories of study participants (years)

The figure 4.1 shows that the ages are categorized into below 56, 56-60, 61-65, 66-70 and above 70. The majority of study subjects were aged below 56 years at 37 %

Table 4.2: The fasting blood sugar (FBS) levels in the study participants

	Gender		Total (n=246)
	Males(n=68)	Females(n=168)	
Mean concentration	10.3	11.7	11
Standard deviation	3.0	3.7	3.3
Range	7.3-13.3	8.0-15.4	7.7-14.3
Minimum concentration	7.1	7.1	7.1
Maximum concentration	21.9	22.8	22.8

* Fasting blood sugar (FBS) unit: mmol/l

The table 4.2 shows that the mean levels of fasting blood sugar in the study participants was 11 mmol/l and the range was from 7.7 to 14.3 mmol/l, minimum and maximum FBS levels were 7.1 and 22.8 mmol/l respectively. The females had both high mean and range concentration compared to the males.

Table 4.3: The glycosylated haemoglobin (HbA1c) levels in the study participants

	Gender		Total (n=246)
	Males (n=68)	**Females(n=178)**	
Mean concentration	8.4	10.6	9.5
Standard deviation	1.8	3.2	2.5
Range	6.6-10.2	7.4-13.6	7.6-12.0
Minimum	6.5	6.5	6.5
Maximum	12.1	20	20

*Glycosylated haemoglobin (HbA1c) unit: %

The table 4.3 shows that the mean levels of Glycosylated haemoglobin (HbA1c) in the study participants was 9.5% and the range varies from 7.0% to 12%. The minimum and maximum Glycosylated haemoglobin (HbA1c) levels were 6.5% and 20 % respectively. The females had both high HBA1c mean and range levels compared to the males.

Table 4.4: Glycosylated Hemoglobin (Hba1c) mean versus Age category

HBA1c category	Ages category (years)					Total
	Below 56	56-60	61-65	61-70	Above 70	
67.9%	25	15	9	3	3	55
	45.5	27.3	16.4	5.5	5.5	100.0
8.51%	20	9	8	3	1	41
	48.8	22.0	19.5	7.3	2.4	100
9.75%	38	22	10	5	4	79
	48.1	27.8	12.7	6.3	5.1	100.0
15.70%	9	17	16	19	10	71
	12.7	23.9	22.5	26.8	14.1	100.0
Total	92	63	43	30	18	246
	37.4	25.6	17.5	12.2	7.3	100.0

The table 4.4 shows that out of the 246 uncontrolled diabetic patients 92 study subjects (37.4%) had age below 56 years while 191 (77.64%) had HBA1c means concentration greater than 8.51%. This was statistically significant since p-value <0.05.

Table 4.5: Glycosylated Hemoglobin (Hba1c) mean versus Gender

HBA1c category	Gender		Total
	Males	**Females**	
67.90%	27	28	55
	49.10%	50.90%	100%
8.51%	12	29	41
	29.30%	70.70%	100%
9.75%	21	58	79
	26.60%	73.40%	100%
15.70%	8	63	71
	11.3	88.7	100%
Total	68	178	246
	27.60%	72.40%	100%

The table 4.5 shows that out of 246, 178 (72.4%) were females and 68 (27.6%) males. The table showed similar percentages of mean HBA1c at 6.79% between females 28 (50.9%) and males 27 (49.1%). This table also showed the predominance of female's percentages 150 (78.53%) to males 41(21.46%) at HBA1c levels from 8.51% to 15.78%. This was statistically significant since p-value=0.000<0.05

Table 4.6: Age category versus diabetes duration categories cross tabulation

Age categories	Diabetes duration categories					Total
	1 – 3	**4 – 6**	**7 – 9**	**10 – 12**	**Above 12**	
Below 56	45	31	8	7	1	92
	48.90%	33.70%	8.70%	7.60%	1.10%	100.00%
56 – 60	26	13	5	11	8	63
	41.30%	20.60%	7.90%	17.50%	12.70%	100.00%
61 – 65	17	6	5	5	10	43
	39.50%	14.00%	11.60%	11.60%	23.30%	100.00%
66 – 70	5	3	2	9	11	30
	16.70%	10.00%	6.70%	30.00%	36.70%	100.00%
Above 70	4	2	1	1	10	18
	22.20%	11.10%	5.60%	5.60%	55.60%	100.00%
Total	97	55	21	33	40	246
	39.40%	22.40%	8.50%	13.40%	16.30%	100.00%

The table 4.6. shows that the age categories are classified as below 56, 56 – 60, 61-70 and above 70. The diabetes duration ranges from 1-3, 4-6, 7-9, 10-12 and above 12 years. The majority of study subjects were classified into the group of below 56 years old 92 (37.4%) and diabetes duration ranging from 1 to 3 years 97(39.4%). The age and diabetes duration categories found statistically significant difference (P<0.05)

Table 4.7: Correlation between glycosylated haemoglobin and fasting blood levels

		Fasting Blood Sugar (mg/dl)	Glycosylated Hemoglobin (%)
Fasting Blood Sugar (mg/dl)	Pearson Correlation	1	.481**
	Sig. (2-tailed)		0
	N	246	246
Glycosylated Hemoglobin (%)	Pearson Correlation	.481**	1
	Sig. (2-tailed)	0	
	N	246	246

Table 4.7 shows a positive(r=0.481) relationship correlation between the Glycosylated Haemoglobin (Hba1c) and Fasting Blood Sugar. The correlation of HBA1c was significantly associated with Fasting Blood Sugar (FBS) levels (p< 0.05).

Table 4.8: Microalbuminuria (MA) levels in the study participants

	Gender		Total
	Males(n=68)	Females(n=168)	(n=246)
Mean concentration	1.3	8.5	5.5
Standard deviation	1.2	16.0	9.2
Range	0.1-2.5	0-24.5	0-14.7
Minimum concentration	0.02	0.04	0.02
Maximum concentration	4.33	97.2	97.2

* Microalbuminuria (MA) unit :mg/dl

The **table 4.8** shows that the microalbuminuria (MA) concentration among the study participants where the mean is 5.5 mg/mmol and the range varies from 0 to 14.7 mg/mmol. The minimum and maximum MA concentration were 0.02 and 92.2 mg/mmol respectively. The females had both high mean and range MA concentration compared to the males.

Table 4.9: Age category versus Microalbuminuria categories cross tabulation

Age categories	Microalbuminuria categories	
	Normal albuminuria (<3mg/mmol)	**Microalbuminuria (>3mg/mmol)**
Below 56	81	11
	32.90%	4.50%
56 - 60	43	20
	17.50%	8.10%
61 - 65	26	17
	10.60%	6.90%
66 - 70	8	22
	3.30%	8.90%
Above 70	7	11
	2.80%	4.50%
Total	165	81
	67.10%	32.90%

The table 4.9 shows that the risk of microalbuminuria (MA) increases with age category where among 32.9% microalbumin positive cases 4.5% belong to less than 56 years and 28.4% belong to above 56 years. It also shows that out of the study population, 165 (67.1%) had norm albumin while (81)32.9% had microalbuminuria and no case of macro albuminuria was recorded. This was statistically significant (p-value <0.05).

Table 4.10: Gender versus Microalbumin categories

Gender	Albuminuria categories		Total
	Normalbumin (≤ 3 mg/mmol)	Microalbumin (>3 mg/mmol)	
Males	55	13	68
%	22.40%	5.30%	27.60%
Females	110	68	178
%	44.70%	27.60%	72.40%
Total	165	81	246
%	67.10%	32.90%	100.00%

The table 4.10 depicts that among 264 uncontrolled diabetic patients 68(27.6%) were males and 178 (72.4%) females. Females were dominant in normalbuminuria and microalbuminuria with 66.7% and 83.9% respectively. This was statistically significant (p-value=0.004 <0.05).

Table 4.11: Diabetes duration categories versus Microalbuminuria categories

Diabetes duration categories	Microalbumin categories		Total
	Normalbuminuria (≤ 3 mg/mmol)	Microalbuminuria (>3 mg/mmol)	
1-3	97	0	97
	100.00%	0.00%	100.00%
4-6	55	0	55
	100.00%	0.00%	100.00%
7-9	13	8	21
	61.90%	38.10%	100.00%
10-12	0	33	33
	0.00%	100.00%	100.00%
Above 12	0	40	40
	0.00%	100.00%	100.00%
Total	165	81	246
	67.10%	32.90%	100.00%

The table 4.11 shows that the risk of microalbuminuria (MA) increases with diabetes duration 1-3,4-6,7-9,10-12 and above 12 with MA of 0%,0%,38.1%,100% and 100% respectively. The diabetes duration showed to be significantly associated with microalbumin (P=0.00 <0.05

Table 4.12: Correlation between glycosylated haemoglobin, microalbumin and diabetes duration

		Micro-albumin (mg/mmol)	Hba1c (%)	Diabetes Duration (Years)
Micro-albumin (mg/mmol)	Pearson Correlation	1	.800[**]	.664[**]
	Sig. (2-tailed)		.000	.000
	N	246	246	246
Glycosylated Haemoglobin (HbA1c) (%)	Pearson Correlation	.800[**]	1	.869[**]
	Sig. (2-tailed)	.000		.000
	N	246	246	246
Diabetes Duration (Years)	Pearson Correlation	.664[**]	.869[**]	1
	Sig. (2-tailed)	.000	.000	
	N	246	246	246

**. Correlation is significant at the 0.01 level (2-tailed).

Table 4.12 shows a very strong positive correlation between Microalbumin and glycosylated haemoglobin HBA1c (r=0.800), microalbumin and diabetes duration (r=0.664), glycosylated haemoglobin and diabetes duration (r=0.869). The correlation was significant at 0.01 (<0.05).
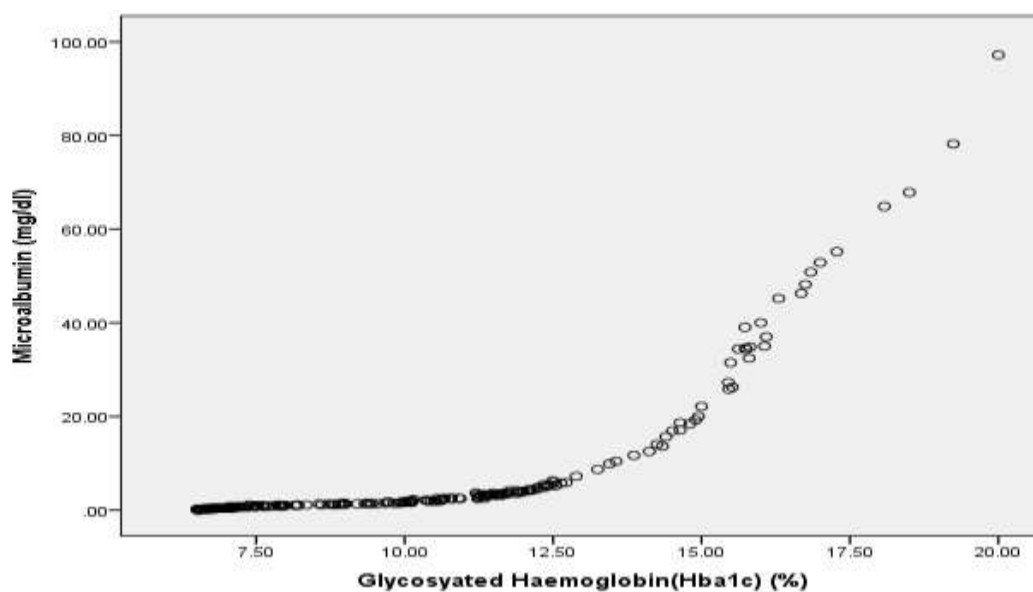
Figure 4.2: Relationship between Glycosylated haemoglobin (HBA1c) and Micro-albumin

A very positive correlation was found between the microalbumin and glycosylated haemoglobin at the level of significance (0.05) and this was evidenced by Pearson correlation coefficient (r=0.800) and (P<0.05) was considered statistically significant.

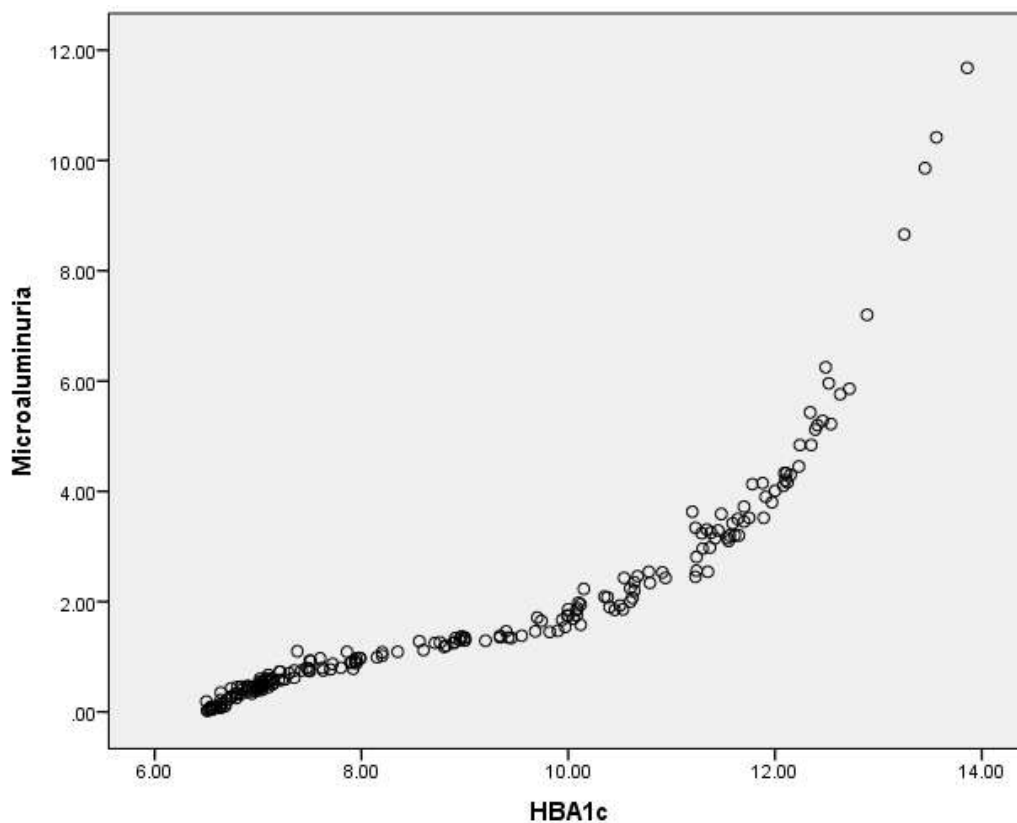**Figure 4.3: Relationship between <14% Glycosylated haemoglobin (HBA1c) and Microalbuminuria levels**

The figure 4.3 shows a very strong positive correlation between the microalbumin and glycosylated haemoglobin this was evidenced by Pearson correlation coefficient (r=0.890) and (P<0.05) was considered statistically significant (P<0.05).
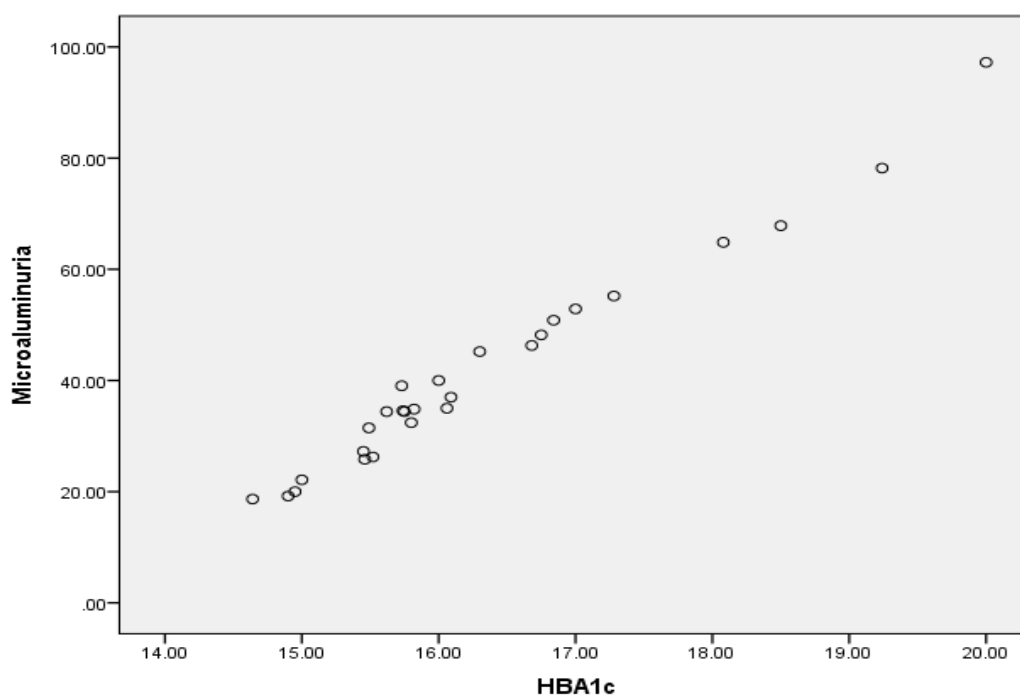
Figure 4.4: Relationship between high (>14%) Glycosylated haemoglobin (HBA1c) and Microalbuminuria levels of the Study Participants

For the patients who had extremely high glycosylated haemoglobin the microalbuminuria is very strongly positive correlated to the glycosylated haemoglobin (HBA1c). It was evidenced by Pearson correlation coefficient (r=0.992) this was considered statistically significant (P<0.05).

Table 4.13: Internal quality control results

	Control level 1		Control level 2	
	HBA1c (%)	Microalbuminuria (mg/mmol)	HBA1c (%)	Microalbuminuria (mg/mmol)
N	30	30	30	30
Target concentration	4.87	2.9	9	13.79
Standard deviation	0.47	0.39	0.57	0.23
Control range	3.8-5.7	2.12-2.78	7.86-10.14	13.33-13.46

The Table 4.13 shows both internal quality control results of HBA1c with the target means and control range. Control 1(normal) had the target means of 4.87% and the control ranges were 3.8% to 5.7% with the confidence interval of 95%. The control level 2 (pathologic control) had the targets means of 9% and pathologic control ranges varied from 7.86-10.14% with the confidence interval of 95%.For microalbuminuria normal control had the target and range of 2.9 and 2.12-2.78 mg/mmol respective. The microalbumuria pathologic control had the target of 13.79 mg/mmol and the range of 13.33-13.46 mg/mmol with the confidence interval of 95%.

HBA1c level 1
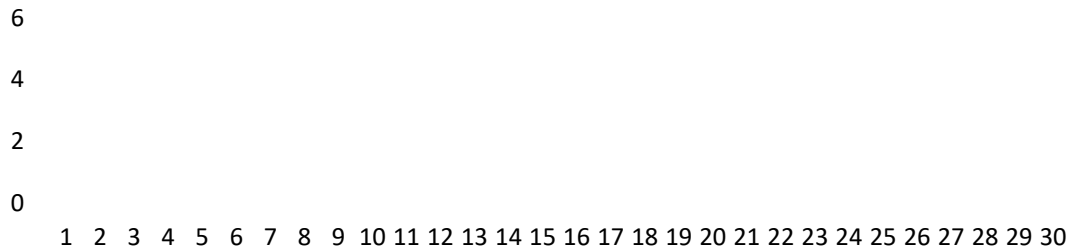
Figure 4.5. Internal quality control (normal HBA1c) Levey Jennings charts

Figure 4.5 shows the control 1(normal) target means of 4.87% and the control ranges were 3.8% to 5.7% with the confidence interval of 95%.
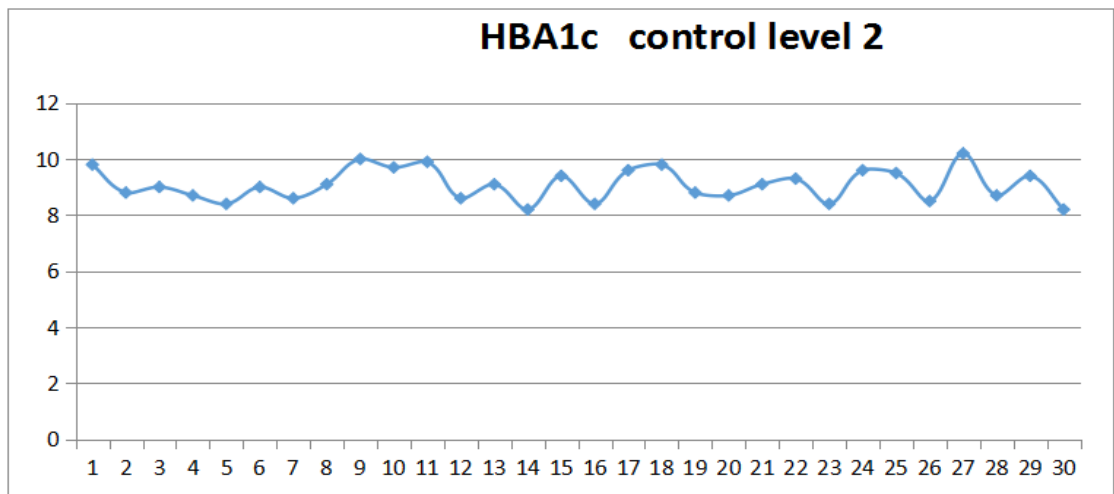
Figure 4.6: Internal quality control (pathologic HBA1c) Levey Jennings charts

Figure 4.6 shows the control level 2 (pathologic control) targets means of 9% and pathologic control ranges varies from 7.86-10.14% with the confidence interval of 95%
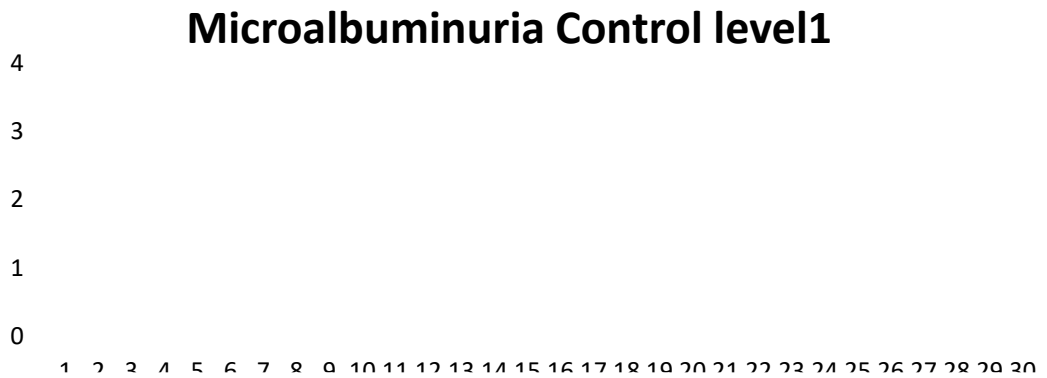
Figure 4.7: Internal quality control (normal microalbuminuria) Levey Jennings charts

Figure 4.7 shows microalbuminuria normal control with the target level of 2.9 and the range varies from 2.12 to 2.78 mg/mmol with the confidence interval of 95%

Microalbuminuria control level 2

```
14.5
 14
13.5
 13
12.5
      1  2  3  4  5  6  7  8  9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
```

Figure 4.8: Internal quality control (pathologic microalbuminuria) Levey Jennings charts

The figure 4.8 shows the microalbumuria pathologic control level of 13.79 mg/mmol and the range of 13.33 to 13.46 mg/mmol with the confidence interval of 95%.

CHAPTER FIVE

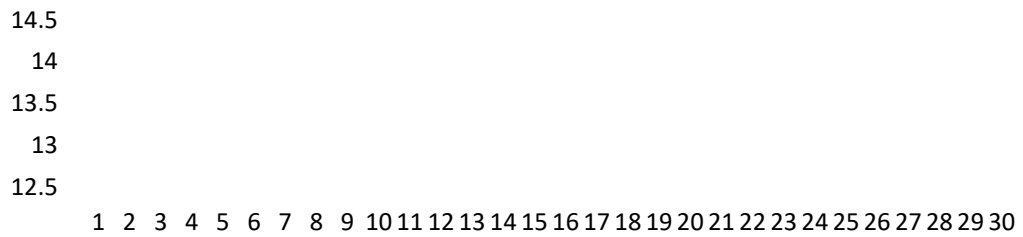## DISCUSSION

Diabetic nephropathy is one of the long-term complications of diabetes mellitus. Shah *et al.,*2017 classified diabetes patients under-treatment based on aggravating glycemic control into normal control (HbA1c < 6.5%) and uncontrolled group (HbA1c > 6.5%). The purpose of this study was to find out the correlation between glycosylated heamoglobin and the microalbumin level among uncontrolled diabetes patients. 246 known uncontrolled type 2 diabetic patients with FBS ≥ 7.0 mmol/l or 126 mg/dL were recruited. In this study there was a females predominance in HBA1c levels category of 8.51% to 15.78% with a of females 78.53% to males 21.46% . The similar study was conducted in Japan by Kajiwara *et al*. 2016 showed that the poor glycemic control increases the risk of diabetic nephropathy in females than in males. The overall prevalence of microalbumin was 32.9% in 246 known uncontrolled diabetes mellitus patients. Different epidemiological and cross-sectional studies have revealed different variations in the prevalence of microalbumin in diabetic patients. Ngassa *et al.,* 2015 reported 23% of microalbuminuria among 798 diabetic patients who had attended the Kalafong Diabetic Clinic (South Africa) in 2012. Wanjohi *et al.*, 2002 reported a microalbumin prevalence of 26% in 100 types 2 diabetic patients in Kenya. Boelter *et al*., 2015 reported the prevalence of microalbumin at 29% in 149 diabetic patients in hospital based-research attending the diabetes clinic at Kilimanjaro Christian Medical Centre in northern Tanzania. Baikai *et al.*, 2018 reported a prevalence of 44.6% among 289 patients with type 2 Diabetes Mellitus in a tertiary clinic in Gaborone, Botswana. The discrepancy in the prevalence of microalbumin can be attributed to several factors such as the difference in population, the sample size, the definition of microalbumin, and the measurement methods of microalbumin and urine collection tested. The all reported prevalence is with a range of 30-40% revealed by Noubiap *et al*., 2015 (b) in the systematic review of diabetic nephropathy in the sub-Saharan Africa region. In the present study, the prevalence of microalbumin among males and females were different percentage 5.3% and 27.6 %, respectively. The higher prevalence of microalbumin among females compared with men was also reported in Nigeria by Okpere *et al.,*2012. The similar observation had re-affirmed by

Chen *et al*., 2014 in prevalence of microalbumin and associated risk factors in china. Gupta *et al.*, 2017 and Geetha & Shanmugasundaram, 2017 also reported increase in microalbuminuria level with age categories and duration of diabetes which has been reaffirmed by this study . This study is in contrast with Omar *et al.*, 2018 have shown the longer duration of diabetes was not associated with poor glycemic control. These variations are probably related to the different distributions of patients' ages in different studies.

The present study has highlighted a strong positive correlation between microalbuminuria with a higher level of HbA1c level (r=0.800, p<0.05) among study participants (r=0.664, p<0.05). There was a statistically significant positive correlation between microalbumin and HBA1c. Ngassa. *et al.,* 2015 reported also a significant positive correlation between HbA1c levels and microalbumin among 754 diabetes patients, attending a diabetes clinic at the Kalafong Hospital in Pretoria, South Africa. A similar correlation was reported by (Subramani & Prabhusamy, 2016) in Velammal medical college hospital, Madurai, a tertiary care hospital (India). Microalbuminuria cases were always confirmed by a second urine specimen in the present study (Treat N *et al*., 2015).

This study agreed with another study done by Muraliswaran *et al*., 2016 in India that found a significant positive correlation between microalbumin and glycosylated haemoglobin in uncontrolled glycemic patients. The positive correlation between microalbuminuria and HbA1c in uncontrolled diabetes mellitus was reported by Khan *et al.*,2012 and in this study, a significant positive correlation between duration of diabetes and HbA1c. A positive correlation between microalbuminuria and duration of diabetes was reported by other previous studies done by Khan *et al*., 2012 in Pakistan, Kamuhabwa & Charles, 2014 in Tanzania and (Idowu *et al*., 2017). Ketema & Kibret, 2015 in a systematic review and meta-analysis on 14 studies revealed a positive correlation between the fasting blood sugar and glycosylated haemoglobin (r=0.28 to 0.84) which is in agreement with our study(r=0.481)

This recognize that the lack of a non-diabetic control population is a challenge of our study; however, this study was not designed to determine the effect of diabetes on the

kidney, but rather to explore the correlation between microalbumin and HBA1c among uncontrolled diabetes mellitus.

5.1 Conclusions

- This study showed that there was a strong correlation (r=0.800) between HBA1c and Microalbumin among the uncontrolled diabetes mellitus type attending Kibagabaga District hospital
- This study showed that the mean levels of Glycosylated haemoglobin (HbA1c) in the study participants was 9.5%
- It also showed that out of the study population, the microalbumin was detected at 32.9% (81/246) while 67.1% (165/246) had no microalbumin.
- This study showed a strong positive correlation between the microalbumin and glycosylated haemoglobin HBA1c (r=0.800), a strong positive correlation between the microalbumin and diabetes duration (r=0.664), very strong positive correlation between the glycosylated haemoglobin and diabetes duration (r=0.869).

5.2 Recommendations

- This study recommends that HBA1c should be inculcated in routine practice for uncontrolled diabetes mellitus patients
- Microalbumin test should be checked at a regular interval: if detected microalbuminuria is detected, confirmation should be made with two further tests within a 3-to-6-month period. If microalbuminuria is not detected, re-screening should be performed annually.
- This study recommended the ministry of Health to implement a guide line for monitoring the uncontrolled diabetes mellitus and the early diabetes nephropathy diagnosis at the district hospitals.

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APPENDICES

Appendix I: Ethical Approval Request Letter

JOMO KENYATTA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY

P.O. BOX 62000-00200 NAIROBI. KENYA. TELEPHONE (067) 52711, 52181/4 FAX: (067) 52164 THIKA

COLLEGE OF HEALTH SCIENCES (COHES)

SCHOOL OF BIOMEDICAL SCIENCES

MEDICAL LABORATORY SCIENCES (MLS) DEPARTMENT

REF: JKU/2/38/007   DATE: 07th December, 2017

TO WHOM I T MAY CONCERN

Dear Sir/Madam,

SUB: SUPPORT LETTER FOR ETHICAL APPROVAL

This support letter is written to Mr. VALENS KARENZI TM 300- 1343/2016 in Medical Laboratory Science **specializing in Clinical Chemistry.**

The above mentioned student has presented a proposal for his master's degree research project to the MLS Department Postgraduate Studies Committee. The title of the project is **"UTILIZATION OF GLYCOSYLATED HAEMOGLOBIN AND MICROALBUMIN IN THE MANAGEMENT OF UNCONTROLLED DIABETES MELLITUS PATIENTS ATTENDING KIBAGABAGA DISTRICT HOSPITAL RWANDA".** The committee has since approved the proposal after appropriate recommended corrections were done on the proposal.

I would like to kindly request that you assist Mr.Karenzi to get ethical clearance **at University of Rwanda (College of Medicine and Health Sciences)** ethics review committee.

For more information, please feel free to contact us.

Yours faithfully,

PROF. JOSEPH GIKUNJU PhD
COD, MEDICAL LABORATORY SCIENCES
E –mail: mls@jkuat.ac.ke

CHAIRMAN
THE MEDICAL LABORATORY
SCIENCE DEPARTMENT
JOMO KENYATTA UNIVERSITY
OF AGRICULTURE & TECHNOLOGY

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Appndix II: IRB Ethical Approval Letter

UNIVERSITY OF RWANDA   COLLEGE OF MEDICINE AND HEALTH SCIENCES

CMHS INSTITUTIONAL REVIEW BOARD (IRB)

Kigali, 3rd /04/2018

KARENZI Valens
JOMO KENYATTA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY

Approval Notice: No 078 /CMHS IRB/2018

Your Project Title *"Utilization Of Glycosylated Haemoglobin And Microalbumin In The Management Of Uncontrolled Diabetes Mellitus Patients Attending Kibagabaga District Hospital Rwanda"* has been evaluated by CMHS Institutional Review Board.

| Name of Members | Institute | Involved in the decision | | |
| | | | No (Reason) | |
		Yes	Absent	Withdrawn from the proceeding
Prof Kato J. Njunwa	UR-CMHS		X	
Prof Jean Bosco Gahutu	UR-CMHS	X		
Dr Brenda Asiimwe-Kateera	UR-CMHS	X		
Prof Ntaganira Joseph	UR-CMHS	X		
Dr Tumusiime K. David	UR-CMHS	X		
Dr Kayonga N. Egide	UR-CMHS	X		
Mr Kanyoni Maurice	UR-CMHS	X		
Prof Munyanshongore Cyprien	UR-CMHS	X		
Mrs Ruzindana Landrine	Kicukiro district		X	
Dr Gishoma Darius	UR-CMHS	X		
Dr Donatilla Mukamana	UR-CMHS	X		
Prof Kyamanywa Patrick	UR-CMHS		X	
Prof Condo Umutesi Jeannine	UR-CMHS		X	
Dr Nyirazinyoye Laetitia	UR-CMHS	X		
Dr Nkeramihigo Emmanuel	UR-CMHS	X		
Sr Maliboli Marie Josee	CHUK	X		
Dr Mudenge Charles	Centre Psycho-Social	X		

After reviewing your protocol during the IRB meeting of where quorum was met on 23rd March 2018, **Approval has been granted to your study.**

EMAIL: researchcenter@ur.ac.rw   P.O. Box: 3286, Kigali, Rwanda   WEBSITE: http://cmhs.ur.ac.rw/ www.ur.ac.rw

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Please note that approval of the protocol and consent form is valid for **12 months**.

You are responsible for fulfilling the following requirements:

1. Changes, amendments, and addenda to the protocol or consent form must be submitted to the committee for review and approval, prior to activation of the changes.
2. Only approved consent forms are to be used in the enrolment of participants.
3. All consent forms signed by subjects should be retained on file. The IRB may conduct audits of all study records, and consent documentation may be part of such audits.
4. A continuing review application must be submitted to the IRB in a timely fashion and before expiry of this approval
5. Failure to submit a continuing review application will result in termination of the study
6. Notify the IRB committee once the study is finished

Sincerely,

Date of Approval: The 3rd April 2018

Expiration date: The 3rd April 2019

Professor Kato J. NJUNWA
Chairperson Institutional Review Board,
College of Medicine and Health Sciences, UR

Ce:
- Principal College of Medicine and Health Sciences, UR
- University Director of Research and Postgraduate Studies, UR

Appendix III: Kibagabaga District Hospital Ethical Approval Letter

REPUBLIC OF RWANDA

Kibagabaga,..2.0.1.0.4./2018

N° 4.6.7../HOP. KIBAG./2018

KIGALI CITY

GASABO DISTRICT

KIBAGABAGA HOSPITAL

PO.BOX 6260 KIGALI

To: KARENZI Valens

RE: APPROVAL TO CONDUCT RESEARCH

Dear sir,

With reference to your letter dated 15th April 2018 requesting research and Ethics committee (IREC) to conduct your research proposal titled: « **Utilization of Glycosylated Hemoglobin and Micro albumin in the management of uncontrolled diabetes mellitus patients attending Kibagabaga District Hospital Rwanda** »

The management is therefore please to inform you that your request had accepted.

Thanks for usual collaboration .

Yours sincerely;

Dr MUTAGANZWA Avite

Director of Kibagabaga District Hospital

Appendix IV: Consent Form

I…………………………………………………agree to participate in study entitled "Utilization of glycosylated haemoglobin and microalbumin in the management of uncontrolled diabetes mellitus patients attending Kibagabaga District Hospital".

The principal investigator 's Mr. Valens KARENZI   Phone: +250788845474

Supervisors: Prof. Jean Bosco Gahutu   Phone: +250783340040

                 Dr. Waithaka King'e   Phone: +254722362719

                 Dr. Amos Mbugua   Phone: +254702961963

I have been provided both written and oral information about the relevant study and have had the opportunity to read the information in peace and quiet and to ask questions.

Through my signature, I agree:

- To participate in the study
- That my information from my medical records (diabetes type, complications, and investigations) may be obtained and used as stated in the written information
- Blood and urine specimens may be collected and be processed.
- Extracts from the interview, medical records, and laboratory results, may be quoted in the thesis and any subsequent publication.

I am aware that my participation is completely voluntary and may discontinue my participation at any time and without further explanation and request that my samples be destroyed without affecting my future care, follow-up, and treatment.

I will also receive a copy of the written information and my consent form.

Please tick one box

I agree to participate in the study

I don't agree to participate in the study

Signature:………………………………………date………../……………./20……

Appendix V: Data Collection Form

DATA COLLECTION FORM

1. Research Id:

2. Age in years:

3. Gender   Male   Female

4. Duration of diabetes: Newly diagnosed

                                        Known case: ....Months

                                                    ....years

5. Fasting Blood Sugar (FBS).........mg/dl

6. HbA1c: ....%

7. Microalbumin (ACR) ....mg/mmol