MICROALBUMINURIA AND SERUM CREATININE IN TYPE 2 DIABETES MELLITUS PATIENTS ATTENDING RENAL CLINIC AT UNIVERSITY TEACHING HOSPITAL IN LUSAKA, ZAMBIA

By

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DECLARATION

I, Shandele Ginnethon Chaamba, do declare that this dissertation represents my own work and that it has neither in part nor in whole, been presented as material for award of any degree at this or any other university. Where other people's work has been used, acknowledgements have been made.

Signed: ……

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APPROVAL

The University of Zambia approves the dissertation of Shandele Ginnethone Chaamba as fulfilling part of the requirements for the award of the degree of Master of Science in Biochemistry (Medical Biochemistry)

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ABSTRACT

The study sought to investigate the presence of Micro-albuminuria (MA) in urine. MA urinary albumin excretion is in the range of 30 mg/24 hrs -300 mg/24 hrs. Consequently, urine albumin excretion has become a key therapeutic target in the management of patients with diabetes mellitus (DM). We aimed at describing the association between Micro-albuminuria, Serum Creatinine and fasting blood glucose in type 2 diabetes mellitus in patients attending Renal Clinic at University Teaching Hospital (UTH).This was an unmatched case control study involving 90 participants from UTH Renal Clinic. The cases included 45 type 2 diabetic patients who met the selection criteria and 45 non-diabetic controls. Blood and Spot urine samples were collected and analyzed for serum creatinine and micro-albuminuria respectively. We found a significant difference in the mean serum creatinine levels between the diabetics (95% CI= 4.231 - 4.438) and controls (95% CI= 3.943-4.163), p= 0.0003. The mean Micro-albuminuria levels between diabetics (95% CI= 3.405 – 3.737) and controls (95% CI = 2.967-3.266), also showed a significant difference, p=0.0001. Serum creatinine showed a weak positive correlation with fasting blood glucose, (r =0.2995 p =0.004) while Micro-albuminuria showed a strong positive correlation with fasting blood glucose, (r =0.3092, p =0.0030). In Zambian setting, both serum creatinine and Micro-albuminuria levels are significantly higher in type 2 diabetes patients compared to controls. Fasting blood glucose levels positively correlated with serum creatinine levels and Micro-albuminuria.

Key words: Micro-albuminuria, Serum Creatinine, Diabetes Mellitus, Fasting blood glucose

DEDICATION

I take the liberty of dedicating this work to my late mother Gail Lupata Shandele (RIP). Her words of excellence, focus and determination still linger deep within my heart. And each time my heart grew weary, I always derived strength to carry on from her wisdom. The mere thought of this woman has always been a source of great academic inspiration. With every word I wrote, it was clear that being better than second best should always be a priority in my life just to always make her proud.

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ACRONYMS / ABBREVIATIONS

MA Micro-albuminuria

SIRS Systemic Inflammatory Response Syndrome

eGFR Estimated Glomerular Filtration Rate

CGM Continuous Glucose Monitoring

DM Diabetes Mellitus

CKD Chronic Kidney Disease

NCDs Non Communicable Diseases

CVD Cardiovascular Disease

DN Diabetic Nephropathy

SSA Sub-Saharan Africa

ROS Reactive Oxygen Species

MFN1 Mitofusin-1

MFN2 Mitofusin-2

OPA Optic Atrophy

FIS 1 Fission-1

DRP1 Dynamin Related Protein-1

MFF Mitochondrial Fission Factor

PVD Peripheral Vascular Disease

mi-RNA Micro RNA

DKD Diabetic Kidney Disease

ESKD	End Stage Kidney Disease
MRI	Magnetic Resonance Imaging
HbA1c	Glycosylated Hemoglobin
KAP	Know Attitude and Practice
ACR	Albumin Creatinine Ratio
UTH	University Teaching hospital
ELISA	Enzyme Linked Immuno-Sorbent Assay
AER	Albumin Excretion Rate
ACEI	Angiotensin Converting Enzyme Inhibitor

CHAPTER ONE

INTRODUCTION

Overview

This chapter gives the background to the study by outlining the main themes. It also presents the statement of the problem, the purpose of the study, the objectives, research questions and limitations of the study. This chapter also provides a detailed explanation of the key concepts in this study and the different mechanisms that lead to micro-albuminuria. It gives a general idea of the impact of diabetes mellitus on kidney function.

1.1 Micro-albuminuria and Serum creatinine

Micro-albuminuria, (MA) represents a condition wherein the urinary albumin excretion is in the range of 30 mg/24 hrs -300 mg/24 hrs (Waghmare and Goswami, 2016). It is considered to be the earliest clinical evidence of diabetic nephropathy (Khadka et al., 2018). Micro-albuminuria, a test easy to perform and of low cost, is a marker of extensive endothelial dysfunction (Barassi et al., 2010). It is associated with increased risk for renal and cardiovascular mortality and morbidity in diabetes mellitus, hypertension, patients with acute myocardial infarction and elderly patients (Verma et al., 2013). Micro-albuminuria is simply a term that can be used to describe a moderate increase in the levels of urine albumin. This usually happens when the kidney leaks small amounts of albumin into the urine, for instance, when an abnormally high permeability for albumin in the glomerulus of the kidney occurs. Under normal circumstances, the kidneys filter albumin, so if albumin is found in the urine, then it means that there could be some kidney diseases. Micro-albuminuria can be considered not only as a risk factor for progressive renal damage, but as providing an integrated assessment of long-term damage to the cardiovascular system, and is therefore increasingly being used in cardiovascular risk assessment clinics (Dawnay, 2014).

It has been central to the development of clinical practice in prevention and treatment of diabetic nephropathy and cardiovascular disease (Parving et al., 2015). It is said to be the only simple, non-invasive, bedside, immediate and cost-effective test to indicate for occurrence of systemic inflammatory response syndrome, (SIRS) if the other variables were stabilized (Emara et al., 2013).

Consequently, urine albumin excretion has become a key therapeutic target in the management of patients with diabetes mellitus (Currie et al., 2014). This is done by measuring the levels of the protein albumin in urine samples. Albumin, is a small globular non-glycosylated protein with a molecular mass of around 66.3 kDa. Its relative abundance along with its high solubility, render it to be an effective carrier in circulation. Suitable pK value (4.6) owing to its charged surface makes it easily dissociable at physiological pH; this factor coupled with its colloidal nature has been contemplated in its significant role for maintenance of plasma oncotic pressure (Waghmare and Goswami, 2016).

Internalization by endocytosis is followed by transport into lysosomes for degradation and the multi-ligand receptors megalin and cubilin are responsible for the constitutive uptake in this mechanism (Gorriz and Martinez-Castelao, 2012). There is a general belief that increased urine albumin excretion in diabetic nephropathy is mostly glomerular in origin.

For albumin to appear in the urine it must cross the glomerular filtration barrier, which consists of fenestrated glomerular endothelial cells, the glomerular basement membrane, and glomerular epithelial cell or podocyte (K. Goud et al., 2012). In a normal kidney, the negatively charged glomerular capillary wall repels negatively charged albumin and prevents its filtration (charge-barrier). Early diabetic pathological changes before the onset of micro-albuminuria are mesangial expansion and glomerular basement membrane thickening (Satirapoj and Adler, 2015). Given this great physiological significance of albumin, teleologically special strategy has been devised to prevent its loss; thus traces of albumin in urine may actually be considered as a mark of deviation from physiological homeostasis (Waghmare and Goswami, 2016).

Measurement of urinary albumin excretion and Estimated Glomerular Filtration Rate (eGFR), has assumed a central role in the diagnosis and management of kidney disease among people with Diabetes and Hypertension (Saha et al., 2015). Although albumin excretion remains the current gold standard marker of glomerular damage in the clinical setting, a number of other proteins have been proposed as useful indicators of early glomerular damage (Currie et al., 2014).

Recently, a model for determining the glucotype of an individual, a more comprehensive measure of the pattern of glucose excursions than the standard laboratory tests in current use was devised. This model is called continuous glucose monitoring (CGM).With greater adoption of CGM technology, glucotype assessment may become an important tool in early identification of those at risk for type 2 diabetes and/or cardiovascular disease (Hall et al., 2018).

Creatinine is the breakdown product of creatine phosphate and is released from skeletal muscle at a steady rate. Serum creatinine correlates quite well with the percent of the body that is skeletal muscle. It is filtered by the glomerulus, and a small amount is also secreted into the glomerular filtrate by the proximal tubule (Bamanikar et al., 2016). An increase in the level of serum creatinine indicates the progression towards diabetic nephropathy and estimation of serum creatinine has greater prognostic ability compared with that of blood urea for predicting the adverse outcomes.

Therefore, increased serum creatinine levels in diabetics clearly indicate prolonged hyperglycaemia which causes irretrievable damage to nephrons of the kidney (Madhusudan et al., 2017). This occurs when there is damage to the kidney or it is not functioning properly.

If the kidneys are unable to function normally, the serum creatinine would not be cleared by the kidneys and would increase abnormally. The elevated levels of HbA1c can be lowered by intensive treatment plan, but the elevated levels of serum creatinine which are set on increase due to permanent damage to the kidneys would be difficult to reverse because damage to the kidneys in diabetes mellitus is a permanent phenomenon. The elevated levels of serum urea and creatinine are the measures of glomerular damage which can, in no way be reversed by intensive treatment plan. The only way to control this progressive glomerular damage and thereby elevated levels of serum creatinine would be early detection and intervention (Chutani and Pande, 2017).

1.2 Diabetes Mellitus and Hypertension

Diabetes Mellitus (DM) can be defined as a group of metabolic diseases characterized by hyperglycemia resulting from defect in insulin secretion, insulin action or even both (Aldukhayel, 2017). Type II diabetes mellitus is a particularly common medical disorder and is leading cause of morbidity worldwide. The complication of DM is due to micro or macro vascular damage (Dhungel et al., 2017).

Diabetes is an increasing problem worldwide; almost 30 million people, nearly 10 percent of the population, in the United States are diagnosed with diabetes. Another 84 million are pre-diabetic, and without intervention, up to 70 percent of these individuals may progress to type 2 diabetes (Hall et al., 2018). Diabetes Mellitus is a steadily growing global epidemic (Janmohamed et al., 2013). Chronic kidney disease (CKD), diabetes, and hypertension play a disproportionate role in the growing public health challenge posed by non-communicable diseases (NCDs) in East Africa (Ploth et al., 2018).

This steady rise is a serious source of concern to the medical fraternity. Sub-Saharan Africa, like the rest of the world, is experiencing an increasing prevalence of diabetes alongside other non-communicable diseases (Hall et al., 2011). On the other hand, hypertension has a high prevalence in the general population, accounts for 1 in 8 consultations in primary care and is a major risk factor for cardiovascular and renal disease.

Despite the wide availability of suitable medicines, only about 25 percent of hypertensive patients have their blood pressure adequately controlled (Bhagani et al., 2018).

It is a common problem in the diabetic population with estimates suggesting a prevalence exceeding 60 percent. Comorbid hypertension and diabetes mellitus are associated with high rates of macro-vascular and micro-vascular complications (Horr and Nissen, 2016). High blood pressure is said to be associated with a 72 percent increase in the risk for all-cause death and a 57 percent increment in the risk for any cardiovascular disease event, making hypertension the strongest driver of cardiovascular outcomes in individuals with diabetes (Cloutier and Lamarre-Cliche, 2018).

Hypertension is basically abnormally high blood pressure in the arteries, which are the blood vessels that carry blood from the heart to the rest of the body. When the heart is beating, it forces blood through the arteries to deliver nutrients and oxygen to the rest of the body. The strength of the blood pushing against the artery walls is blood pressure, which is measured in units called millimeters of mercury (mmHg). This is expressed using a fraction of numbers, one on top (the numerator) and the other below (the denominator). The top number in a blood pressure reading is the pressure when the heart pumps (systolic blood pressure), and the bottom number is the pressure between heart beats (diastolic blood pressure). In adults, a normal blood pressure measurement is about 120/80 mmHg (Pavlou et al., 2018). Blood pressure is considered high when the measurement is 130/80 mmHg or greater.

Hypertension is twice as frequent in patients with diabetes compared with those who do not have diabetes (Petrie et al., 2018). It is present in the majority of people with type 2 diabetes mellitus and both micro-vascular (retinopathy and albuminuria) and macro-vascular (myocardial infarction and stroke) complications are significantly more common in patients with combination of diabetes and hypertension than in those without hypertension (Niakan and Cushman, 2018). Hypertension usually has no symptoms, and many affected individuals do not know they have the condition. However, hypertension is a major risk factor for heart disease, stroke, kidney failure, and eye problems. When blood pressure is elevated, the heart and arteries have to work harder than normal to pump blood through the body. This causes a lot of serious risks to one's health. The extra work thickens the muscles of the heart and arteries and hardens or damages artery walls. As a result, the flow of blood and oxygen to the heart and other organs is reduced. Damage to the heart caused by the extra work and a lack of oxygen causes heart disease. In addition, damage to the arteries increases the risk of blood clots that block the flow of blood to the heart, causing a heart attack, or to the brain, causing a type of stroke known as an ischemic stroke.

It has been estimated that two-thirds of patients with type 2 diabetes mellitus have arterial hypertension. This increases the incidence of both micro- and macro-vascular complications in these patients, while the co-existence of these two major risk factors leads to a four-fold increased risk for cardiovascular disease (CVD) compared with normotensive non-diabetic controls (Pavlou et al., 2018).

Another type of stroke, called a hemorrhagic stroke, can occur when a weakened blood vessel in the brain bursts. Damage to blood vessels in the kidneys impairs their ability to filter waste and remove fluid, leading to kidney failure. In about 95 percent of cases, the cause of hypertension is unknown. These cases are classified as essential hypertension. When hypertension results from an underlying condition, such as blood vessel defects that reduce blood flow; kidney disorders, which alter the amount of fluids and salts in the body; or problems with hormone-producing glands called the adrenal glands or the thyroid gland, it is classified as secondary hypertension. This combination establishes the fact that hypertension and diabetes mellitus are also stroke risk factors and correlated in patients with atherosclerosis (Alloubani et al., 2018). It must also be noted that people with controlled diabetes have a similar cardiovascular risk to patients without diabetes but with hypertension (Ferrannini and Cushman, 2012).

1.3 Systolic Blood Pressure and Fasting Blood Glucose

The alteration of circadian blood pressure appears to precede the micro-albuminuria: in longitudinal studies there is evidence that an increase in systolic blood pressure during sleep precedes the development of micro-albuminuria (Barbieri et al., 2016). Furthermore, an increase in blood pressure during morning period, detected by ambulatory blood pressure monitoring, preceded the development of micro-albuminuria.

Thus, the risk of micro-albuminuria, a marker of kidney disease in patients with type 2 diabetes, appears to be very low in patients who remain normotensive, as defined not only by normal blood pressure readings at office visits and during ambulatory daytime monitoring over time but also by the absence of an increase in systolic pressure during the morning period.

Increased Blood Pressure: is a major determinant in the progression towards end-stage renal failure. Numerous cross sectional studies have shown that micro-albuminuria is associated with the increased blood pressure (Muhammad et al., 2017). More commonly, systolic blood pressure has a positive influence on the progression of micro-albuminuria. It is well established that overt proteinuria in diabetes is associated with elevated blood pressure and early mortality.

Interestingly, obesity also has become increasingly recognized as a risk factor for kidney disease and both proteinuria and micro-albuminuria have been associated with obesity. Furthermore, obesity is associated with risk factors of kidney disease, such as diabetes and hypertension (HTN), and the prevalence of proteinuria and albuminuria excluding these risk factors is uncertain. In developing countries, the nutritional status has improved along with the economic growth. A number of studies have provided strong evidence of an association between patients who are overweight or obese and risk of DM. Glycemic control plays as vital role in diabetic micro-vascular complications. Intensive glycemic control can have certain benefits on prevention of diabetic nephropathy in patients with high cardiovascular risks. Therefore, the implementation of glycemic control may have different benefits affecting different stages of diabetic nephropathy (Chen et al., 2014). One study found that the odds ratio for micro-albuminuria increases significantly from the stage of high-normal blood pressure and impaired fasting glucose level compared with their reference categories (Munakata et al., 2011). These data provide very important suggestions about the stage at which intervention should be initiated.

1.4 Diabetic Nephropathy

Diabetic Nephropathy (DN) is a common and serious complication of diabetes associated with adverse outcomes of renal failure, cardiovascular disease, and mortality (Fiseha and Tamir, 2016). Diabetic nephropathy (DN) is characterized by albuminuria, which is usually accompanied by hypertension, progressive rise in proteinuria (Saxena, 2014).

Moderately increased albuminuria is widely accepted as the first clinical sign of diabetic nephropathy (Said and Nasr, 2016). It is said to be the leading cause of end-stage renal disease in the world (Tahara and Takasu, 2018).

Diabetic nephropathy is a major underlying cause of morbidity and mortality in both type 1 and type 2 diabetes mellitus, giving rise principally to cardiovascular disease, in particular heart failure, the incidence of which is about 15-fold greater in patients with diabetic kidney disease (Thomas and Karalliedde, 2015).

It is one of the most prevalent lethal complications of diabetes that leads to end stage renal disease (Dewanjee and Bhattacharjee, 2018). It is a clinical syndrome characterized by persistent albuminuria (> 300 mg/24 h, or 300 mg/g creatinine), a relentless decline in glomerular filtration rate, raised arterial blood pressure and enhanced cardiovascular morbidity and mortality (Rossing et al., 2018).

Diabetic Nephropathy caused by hypertension and unmitigated inflammation in diabetics, renders the kidneys unable to perform normally, and leads to renal fibrosis and organ failure (Brenneman et al., 2016).About 415 million people worldwide had diabetes in 2015. The global figure of people with diabetes is projected to increase to 642 million by 2040. As the prevalence of diabetes has risen to epidemic proportions worldwide, diabetic nephropathy has become one of the most challenging health problems (Fukami and Yamagishi, 2018). It is estimated that more than 1 in 3 people with type 2 diabetes have impaired kidney function (Eboh and Chowdhury, 2015). Diabetic kidney disease and diabetic nephropathy are the leading cause of end-stage kidney disease in the United States and most developed countries.

Although this represents a significant public health concern, it is important to note that only 30 percent to 40 percent of patients with diabetes develop diabetic nephropathy (Umanath and Lewis, 2018).

Even if various therapeutic approaches, such as hypoglycemic agents, antihypertensive drugs, and renin-angiotensin system inhibitors, have been tried to slow the progression of nephropathy, the number of patients with diabetic kidney disease continues to rise with the prevalence of type 2 diabetes mellitus (John, 2016).

It must be noted, however, that currently, available therapies provide only symptomatic relief and unable to treat the underlying pathophysiology of diabetic nephropathy (Sharma et al., 2017). It is well appreciated both that coexisting hypertension exacerbates diabetic nephropathy and that diabetic nephropathy somehow results in a markedly increased risk of hypertension (Ali et al., 2014). A great deal of research has since been conducted on diabetes mellitus worldwide. A recent study shows that, there is uncertainty what really contributes to the development of micro-albuminuria in type 2 diabetes (Barbieri et al., 2016).

Another study indicated that, there is an alarming high prevalence of chronic kidney disease among Tanzanian adult diabetics (Janmohamed et al., 2013). Furthermore, recent research on this subject suggests that, tubular injury, as shown by increased urinary tubular damage markers at the micro-albuminuria stage of diabetes, is a critical component of the early course of diabetic nephropathy (Fiseha and Tamir, 2016). Even with great effort by many researchers worldwide, the situation with regard to diabetes mellitus and micro-albuminuria still remains a burden which is on the rise (Hall et al., 2011). One reason why this still remains a burden is that, currently there is no proper understanding of the mechanisms that may contribute to microalbuminuria in type 2 diabetes (Barbieri et al., 2016). This may be due to lack of adequate information on the subject. Hence, it becomes difficult to find correct and prompt measures to prevent or even slow down the possible future complications that are associated with prolonged type 2 diabetes mellitus.

1.5 Study Justification

There is a high burden of micro-vascular complications in patients with type 2 diabetes (Olamoyegun et al., 2015). The morbidity and mortality due to diabetes mellitus at the UTH was 561 (7.7 percent) and 114 (20.3 percent) respectively in 2010 (Musenge et al., 2014). In recent years, one study showed an assessment of albuminuria in rural Sub-Saharan Africa and was conducted in rural Zambia. However, no association was measured in the study mentioned above. It is very important to understand the impact type 2 diabetes mellitus may have and the eventual complications that follow if timely interventions are not made. Many diabetic patients face significant challenges accessing diagnosis and treatment, which contributes to the high mortality and prevalence of complications observed (Hall et al., 2011). To alleviate the several challenges, correct information must be availed to the necessary authorities through studies such as this one. Additionally, complications of diabetes mellitus are more prevalent among patients with diabetes in Africa as compared to the developed world due to late presentation, limited screening and diagnostic resources, poor glycemic control, and inadequate treatment of complications at an early stage (Janmohamed et al., 2013).

If the relationship between micro-albuminuria and serum creatinine levels in type 2 diabetes mellitus is well established, then clearly, effective early detection and screening can be done.

The main focus of this study therefore, is to establish how the micro-albuminuria and serum creatinine levels compare in diabetics and non-diabetics. The study further analyzes the difference in levels of micro-albuminuria and serum creatinine between diabetics with hypertension and those without hypertension. It also gives a description of the correlation between fasting blood glucose levels and micro-albuminuria as well as serum creatinine to help better understand diabetes mellitus and the associated risk factors. This could eventually lead to improved clinical outcomes. With improved clinical outcomes, the progression of type 2 diabetes mellitus to complications such as end stage renal disease will be slowed down. A certain study showed that the lesions of diabetic nephropathy are reversible and that the kidney can undergo substantial architectural remodeling upon long-term normalization of the diabetic mellitus (Fioretto et al., 2014). Thus, the results obtained from this study may possibly help in management of patients with type 2 diabetes mellitus.

1.6 Problem Statement

Diabetes Mellitus remains a tremendous challenge to public health worldwide (Ali et al., 2014). On average, 20-40 percent of patients with diabetes mellitus will develop renal dysfunction (Al-Rubeaan et al., 2014).With such diverse health challenges like renal dysfunction, health authorities in Sub-Saharan Africa and international donors need robust data on the epidemiology and impact of diabetes in order to plan and prioritize their health programs (Hall et al., 2011).

If information on micro-albuminuria and type 2 diabetes mellitus is not adequate enough, diabetes mellitus can progress to diabetic nephropathy and eventually end stage renal disease (Fiseha and Tamir, 2016). In sub-Saharan Africa, such renal complications of diabetes may go unrecognized due to limited diagnostic resources (Janmohamed et al., 2013). In order to prevent or even slow down the progression to end stage renal disease in diabetes mellitus patients, it is of great importance to have early screening and diagnostic resources for diabetes mellitus patients to institute correct and timely interventions. The ability to screen on time, give correct diagnosis

and suggest accurate interventions largely depends on availability of necessary information. This study is aimed at establishing the correlation between micro-albuminuria, serum creatinine and fasting blood glucose levels in type 2 diabetes mellitus patients.

From current medical literature, it is unclear; whether micro-albuminuria is related to diabetes mellitus (Barbieri *et al*., 2016). From the above problem statement, the following research question was raised.

1.7 Research Question

What is the relationship between micro-albuminuria, serum creatinine levels and fasting blood glucose levels in type 2 diabetes mellitus patients attending renal clinic at University Teaching Hospital in Lusaka, Zambia.

1.8 General Objective

To determine the correlation between micro-albuminuria, serum creatinine and fasting blood glucose levels in type 2 diabetes mellitus patients attending renal clinic at University Teaching Hospital, in Lusaka, Zambia.

1.9 Specific objectives

1. To determine the mean micro-albuminuria and serum creatinine levels in diabetic and non-diabetic participants attending renal clinic at the University Teaching Hospital, in Lusaka, Zambia.
2. To establish the relationship between micro-albuminuria and serum creatinine levels in diabetes mellitus patients attending renal clinic at University Teaching Hospital, in Lusaka Zambia.
3. To determine the correlation between micro-albuminuria, serum creatinine levels and fasting glucose levels in diabetes mellitus patients attending renal clinic at University Teaching Hospital in Lusaka, Zambia.

1.9.1 Limitations

This research had a number of limitations. Firstly, the age of most diabetic participants could have confounded the study as duration of diabetes is critical in the excretion of urine albumin. The duration of diabetics was not indicated in the patients file and so it was difficult to know how long one has lived with diabetes. This would have been important in trying to understand how duration of diabetes would affect the excretion of urine albumin

1.9.2 Organization of dissertation

This chapter gives background information of the study. It explains the meaning of micro-albuminuria, serum creatinine, and diabetes mellitus. It contains the study justification, problem statement, research questions and objectives as well as the limitations of the study. Chapter two is a detailed analysis of the literature that was revealed throughout this study. In this chapter, different research outcomes have been brought forward and explained. Chapter three is a step-wise explanation of what was done to arrive at the observations made in the research. It shows the population chosen, the selection criteria, the study design used and the inclusion and exclusion criteria. It shows how clinical data was collected and analyzed to have a clear understanding. It also shows the tools used in analyzing the given data. Chapter four contains the presentation of findings. These are results of the experiments conducted and what they mean. Chapter five is the discussion of the findings. This gives a critical explanation of why the observations seen were actually made in this study and how these findings compare with other similar studies. Furthermore, it also gives scientific explanations as to why the observed results could have been as seen. In chapter six, a conclusion will be given as well as recommendations to help in the making of policies that can thus improve clinical outcomes.

Summary

This chapter gave the background to the study by outlining the main themes. It also presented the statement of the problem, the purpose of the study, the objectives and research questions. It created a clear understanding of diabetes mellitus and its risk factors. This chapter provided a detailed explanation of the key concepts in this study that will make the following chapters a little easy to understand.

CHAPTER TWO

REVIEW OF RELATED LITERATURE

Overview

This chapter reviews related literature on micro-albuminuria in type 2 diabetes mellitus as well as non-diabetic participants. It gives a detailed background on micro-albuminuria, serum creatinine levels, the possible causes, and the other risk factors associated with it. It also explains the pathophysiology of diabetes mellitus. In addition, it will critically compare what has been done, internationally on the subject matter with the local observations.

2.1. Case definition of Diabetes Mellitus

The global incidence of metabolic disorders like type 2 diabetes mellitus has assumed epidemic proportions, leading to adverse health and socio-economic impacts. Type 2 diabetes mellitus is a serious and lifelong condition commonly characterized by abnormally elevated blood glucose levels due to a failure in insulin production or a decrease in insulin sensitivity and function (Tan et al., 2018). Diabetes is one of the concerns of today's public health and patients with type 2 diabetes are at increased risk of death due to cardiovascular diseases (Saed et al., 2019). Type 2 diabetes mellitus is a major public health challenge that affects countries across the world (Yan and Khalil, 2017).

The prevalence of type 2 diabetes mellitus has been increasing worldwide. Due to its continuously increasing occurrence, more and more families are influenced by diabetes mellitus (Wu et al., 2018).Cardiovascular diseases are one of the main causes of death among people with type 2 diabetes mellitus (Loyola-Leyva et al., 2018). Type 2 diabetes mellitus is a growing concern worldwide, particularly in Indigenous communities, which have undergone a marked nutrition transition characterized by reduced intakes of traditional foods and increased intakes of market foods (Reeds et al., 2016).

It constitutes a global health threat, with increasing burden of disease in low and middle-income countries witnessing ongoing epidemiological transition (Eltom et al., 2018). Compared to global estimates, Sub-Saharan Africa (SSA) has the highest projected rates of increase in type 2 diabetes over the next 25 years. This obviously is attributed to the ageing population, increasing urbanization and the associated lifestyle changes (Goedecke et al., 2017).

The burden of uncontrolled type-2 diabetes mellitus in sub-Saharan Africa is high, with an increased risk of developing micro-vascular and macro-vascular complications (Mobula et al., 2018). Diabetes mellitus type 2 has been associated with an increased cardiovascular risk. Improving glycaemia or other traditional cardiovascular risk factors may reduce cardiovascular risk in patients with type 2 diabetes mellitus (Burggraaf and Castro Cabezas, 2017).

The multifactorial etiology of type 2 diabetes mellitus is relative to many gene and molecule alterations, and increased insulin resistance. Besides these, however, there are still other predisposing and risk factors accounting for type 2 diabetes mellitus not yet identified and recognized (Fang et al., 2016).

Most of diabetes mellitus patients are affected by type 2 diabetes mellitus with insulin resistance and insulin secretion defect (Xu et al., 2018).

It must be noted that type 2 diabetes mellitus in adolescents, those between the ages of 12 and 18 years, has also gone from unusual to increasingly common. This rise in prevalence is attributed to the increase in pediatric and adolescent obesity (Zappas and Granger, 2017). It is believed that two-thirds of patients with type 2 diabetes mellitus have arterial hypertension. This increases the incidence of both micro- and macro-vascular complications in these patients, while the co-existence of these two major risk factors leads to a four-fold increased risk for cardiovascular disease (CVD) compared with normotensive non-diabetic controls (Pavlou et al., 2018).

2.2 Prevalence of Diabetes Mellitus

About 415 million people live with diabetes worldwide, and an estimated 193 million people have undiagnosed diabetes. Type 2 diabetes accounts for more than 90 percent of patients with diabetes and leads to micro-vascular and macro-vascular complications. Despite increasing knowledge regarding risk factors for type 2 diabetes mellitus and evidence for successful prevention programs, the incidence and prevalence of the disease continues to rise globally. Young (Chatterjee et al., 2017). The prevalence of diabetes around the world has reached epidemic proportions. While diabetes is already estimated to affect more than 8 percent of the global population (nearly more than 350 million people), this is predicted to grow to over 550 million people by the year 2035 (Gheith et al., 2015). Some studies have shown that the prevalence of type 2 diabetes mellitus increases markedly with age (Gómez-Huelgas et al., 2018).

Diabetic nephropathy is also a leading cause of end stage renal disease and in combination with the increasing worldwide prevalence of diabetes poses an enormous burden to healthcare systems (Currie et al., 2014). Not only is this a western challenge, with increasing prevalence and interactions with other diseases, including the major communicable diseases of the region, diabetes is becoming a pressing public health problem for Sub-Saharan Africa (Hall et al., 2011). The current state of affairs therefore, dictates that aggressive studies on type 2 diabetes mellitus should be conducted worldwide. This is particularly important in sub-Saharan Africa where few treatment options exist for end stage renal disease, and it will become even more important as the incidence of diabetes continues to increase in this region (Janmohamed et al., 2013). It is critical for Sub-saharan Africa and especially Zambia in particular where such studies are scarce.

2.3. Pathophysiology and risk factors of diabetes mellitus

Type 2 diabetes mellitus is a disease involving both inadequate insulin levels and increased glucagon levels. While glucagon and insulin work together to achieve optimal plasma glucose concentrations in healthy individuals, the usual regulatory balance between these 2 critical pancreatic hormones is awry in patients with diabetes (Hædersdal et al., 2018).

The prevalence of Diabetes Mellitus Type 2 is increasing every passing year due to some global changes in lifestyles of people. The exact underlying mechanisms of the progression of this disease are not yet known. However recent advances in the combined omics more particularly in proteomics and genomics have opened a gateway towards the understanding of predetermined genetic factors, progression, complications and treatment of this disease (Sohail et al., 2018).

Randomized trials report that a proportion of individuals with pre-diabetes develop diabetes despite caloric restriction, physical activity, and/or when treated with metformin, the first-line medication for patients with type 2 diabetes mellitus (Samocha-Bonet et al., 2018). Type 2 diabetes mellitus is characterized by mitochondrial dysfunction, high production of reactive oxygen species (ROS) and low levels of ATP.

Mitochondrial fusion is modulated by different proteins, including mitofusin-1 (MFN1), mitofusin-2 (MFN2) and optic atrophy (OPA-1), while fission is controlled by mitochondrial fission 1 (FIS1), dynamin-related protein 1 (DRP1) and mitochondrial fission factor (MFF) (Rovira-Llopis et al., 2017).

Furthermore, accelerated atherosclerosis in patients with diabetes mellitus contributes an increased risk of developing cardiovascular diseases including peripheral vascular disease (PVD) (Dryden et al., 2015).

Recent studies have pointed out that specific micro-RNA (miRNAs) play a critical role in controlling β cell activities and the development of diabetic vascular complications. The association of micro-RNA with the disease pathogenesis and omnipresence in body fluids have made them important players for prognosis, diagnosis and management of type 2 diabetes mellitus (Banerjee et al., 2017). Genome-wide association studies have also implicated around 250 genomic regions in predisposition to type 2 diabetes mellitus, with evidence for causal variants and genes emerging for several of these regions (Langenberg and Lotta, 2018). Further results suggest that alpha-cell dysfunction precedes the type 2 diabetes mellitus development. This process seems to be independent of diet consumed (Roncero-Ramos et al., 2018). Type 2 diabetes results from interplay between genetic and acquired factors. Glycans on proteins reflect genetic, metabolic and environmental factors (Lemmers et al., 2017).

Obesity and being overweight is the most powerful risk factor accounting for 80–90 percent of patients with type 2 diabetes mellitus. The epidemic of obesity is driving the diabetes epidemic to alarming levels and primary care is becoming an important setting for obesity management in type 2 diabetes mellitus (Mohammad and Ahmad, 2016). Type 2 diabetes mellitus is also a significant risk factor for developing Alzheimer's disease later in life, and particular populations have a disproportionate risk because of the high prevalence of type 2 diabetes. There are many overlapping pathologies, and teasing out the primary root cause, if one indeed exists, is very difficult (Silzer and Phillips, 2018). It is therefore of critical importance to have early diagnosis of type 2 diabetes patients and the detection of those at increased risk of disease (Kraniotou et al., 2018).

2.4. Complications and Management of Diabetes Mellitus

Type 2 diabetes mellitus is a growing pandemic that will lead, if not managed and controlled, to frequent complications, poor quality of life, and high rates of disability and death (Ghandour et al., 2018). Diabetic kidney disease (DKD) is a thoughtful complication that takes place in 20 percent to 40 percent of all diabetics in the Western world and that diabetic kidney disease is the primary single cause of end-stage kidney disease (ESKD) (Gheith et al., 2015). It has been noted that Chronic kidney disease (CKD) is one of the most common complications of diabetes mellitus (Janmohamed et al., 2013). Both type 1 and type 2 diabetes can lead to nephropathy, but in type 2 diabetes, a smaller proportion of the patient’s progress to ESKD.

Because of higher prevalence of type 2 diabetes, these patients represent more than half of diabetics on hemodialysis (Gheith et al., 2015). It is well appreciated both that coexisting diabetes mellitus and micro-albuminuria exacerbates diabetic nephropathy and that diabetic nephropathy somehow results in a markedly increased risk of hypertension (Ali et al., 2014). Many theories have been put across to try and explain the current complications of diabetes. In one study it was established that Glomerular damage increases permeability to plasma proteins resulting in their excretion in the urine (Currie et al., 2014). Obviously, this Glomerular damage is as a result of poor glycemic control leading to consistent high levels of blood glucose.

In addition, abnormalities of extracellular matrix synthesis and degradation in kidney disease can lead to increased urinary excretion of matrix proteins, reflecting glomerular injury (Currie et al., 2014).

The major complications related to diabetes are mostly due to the macro-vascular and micro-vascular bed impairment due to metabolic, hemodynamic and inflammatory factors (Visca et al., 2018). Functional and structural magnetic resonance imaging (MRIs) have demonstrated that patients with type 2 diabetes mellitus exhibit abnormalities in brain regions in the cerebral cortex (Fang et al., 2017). Epidemiologic and clinical data from the last 2 decades have shown that the prevalence of heart failure in diabetes is very high, and the prognosis for patients with heart failure is worse in those with diabetes than in those without diabetes (Lehrke and Marx, 2017).

Worldwide, an estimated 200 million people have chronic kidney disease (CKD), whose most common causes include hypertension, arteriosclerosis, and diabetes. Diabetes mellitus is a chronic disease which has been associated with depression. Depression is more common in adults with type 2 diabetes mellitus as compared to those without. Since Type 2 diabetes mellitus is a global health-care and national policy issue. As fluctuating glycemic control in diabetes often results in serious complications, we must encourage the diabetes educators' efforts at long-term follow-up among patients with type 2 diabetes (Wang et al., 2014). Additionally, chronic pressure overload conditions are highly prevalent amongst diabetic population and this association leads to a more severe myocardial impairment (Gonçalves et al., 2016).

The last decade has seen a radical change in our assumptions about the best ways to lower cardiovascular risk in patients with type 2 diabetes mellitus. There is now overwhelming evidence that lowering cholesterol with statin therapy and lowering blood pressure with antihypertensive agents, at least to a systolic value of 130 mmHg, are the keys to success in achieving such benefits (Preiss and Sattar, 2015). Further research shows that the Internet-based U-Healthcare system of integrated management in diabetes not only achieved better glycemic control, effectively improved glycosylated hemoglobin, (HbA1c) levels and decreased triglyceride levels but also enhanced patients' adherence to the medical team's instructions (Wang et al., 2017). Above all, diabetes knowledge has been shown to improve glycemic control and associate with several demographic parameters, (Poulimeneas et al., 2016).

The knowledge, attitude, and practice (KAP) are vital in diabetes management (Abbasi et al., 2018). Dietary and physical activity advice have been considered to be seminal ingredients in prevention and management of type 2 diabetes mellitus (Abdulah et al., 2018).

Proper management of type 2 diabetes mellitus is very important as it helps in slowing down the progression to conditions such as diabetic nephropathy. Prevention of nephropathy can be achieved by tight glycemic and blood pressure control (Eboh and Chowdhury, 2015).

Research suggests that, tubular injury, as shown by increased urinary tubular damage markers at the micro-albuminuria stage of diabetes, is a critical component of the early course of diabetic nephropathy (Fiseha and Tamir, 2016).

Appropriate large scale, multicenter studies are needed to generate reliable evidence on the prevalence and predictors of micro-albuminuria even in children and adolescents with elevated BP (Flynn, 2016). There is also need to devise new, accurate, easy-to-use, and inexpensive ways to identify patients at risk (Wahab et al., 2017). And in some studies it was noted that, higher urinary albumin creatinine ratio (ACR) levels in adults with type 2 diabetes were associated with a greater incidence of macro-albuminuria, after excluding patients with clear-cut evidence of non-diabetic kidney diseases (Chida et al., 2016).

The presence of micro-albuminuria is an indication to the physician to take steps to prevent further renal damage by correction of risk factors, such as control of diabetes and hypertension (Aldukhayel, 2017). The only way a physician will be able to make correct decisions is when they are guided by overwhelming evidence suggesting that such measures should be put in place. In the last 10 years, new lines of research have emerged for the treatment of type 2 diabetes mellitus and preclinical studies appear promising. The possibility of using these drugs in combination with other currently available drugs will enhance the anti-diabetic effect and promote weight loss with fewer side effects (Puig-Domingo and Pellitero, 2015).

2.5 Mechanisms of Glomerular Filtration

The glomerular basement membrane (GBM) is an integral component of the glomerular filtration barrier; an important and highly complex capillary wall that is exposed to mechanical forces driven by capillary hydrostatic pressure. This barrier is permeable to water and small molecules, and selectively withholds cells and macromolecules such as albumin in the circulation (Chew and Lennon, 2018). These characteristics are dependent on its unique three-layer structure (Satchell, 2012). The filtration barrier consists of sequentially finer filters, beginning with the endothelium, then the basement membrane, and then the renal podocytes and their slit diaphragms (Feher, 2017). A defect in or injury to any one of these three components can cause pathologic leak of albumin and other plasma proteins into the urine. When the protein albumin finds itself in urine, it therefore becomes a clear indication that damage has been done on the blood vessels within the kidneys such that proteins can easily leak out of the filtration slits. This is what eventually leads to micro-albuminuria. This and a wealth of other evidence suggest that the three layers interact to establish and maintain the glomerular filtration barrier to plasma macromolecules (Goldberg et al., 2010).

The three fundamental operations of the kidney are ultrafiltration, reabsorption, and secretion, and all three are performed in miniature by each nephron. The filtration barrier is a target of injury in several systemic and renal diseases, and this often leads to progressive renal disease and kidney failure (Patrakka and Tryggvason, 2010).

The glomerular volume, fractional interstitial area, fractional mesangial area, and GBM width are all higher in diabetes and tend to increase with increasing albuminuria. Diabetics have a reduced filtration surface area density, while the total surface area is increased in micro-albuminuria (Satchell, 2012).

Summary

This chapter reviewed related literature on micro-albuminuria in type 2 diabetes mellitus as well as non-diabetic participants. It gave a detailed background on micro-albuminuria, serum creatinine levels, the possible causes, and the other risk factors associated with it. It also explained the pathophysiology of diabetes mellitus, micro-albuminuria and serum creatinine. This section gave a greater understanding of what other researchers have done.

CHAPTER THREE

METHODOLOGY

Overview

This chapter explores the methods used in this research. It clearly explains the techniques used in sampling and also setting up of the study. This section also has the inclusion and exclusion criteria. The procedures used to carry out each particular stage of this research have been clearly explained in this section. The collection of data is also outlined as well as the analysis of the data that was collected.

3.1 Study Design

This was an unmatched case control study in which eligible type 2 diabetes mellitus patients at UTH renal clinic were enrolled to the study after consent.

3.2 .Study Setting

The study was conducted at the renal clinic at the University Teaching Hospital in Lusaka. Being the largest referral hospital in the country, a good number of outpatients visit this place from all over the country in search for review and treatment. The hospital receives referral patients from districts and provincial hospitals from all over Zambia and from the health centers that are in Lusaka. The renal clinic at UTH is a specialist clinic consisting of patients with different non-communicable disease that have been referred for further management. Diabetes mellitus patients are reviewed once every week on Tuesdays.

3.3 Target and Study Populations

The target population was confirmed type 2 diabetes mellitus patients attending out-patients renal unit at the University Teaching Hospital who were 18 years and above.

The study population was confirmed type 2 diabetes mellitus patients who met the inclusion criteria and provided written consent to the study.

3.4 Sample Size

Using the epiinfo software, a total sample size of 97 enrolled but only 90 participated in the study. The 7 were excluded because they could not give consent to the study. This sample size included 45 type 2 diabetes mellitus patients and 45 individuals without diabetes mellitus (control group). This sample size was arrived at using an expected frequency of 12.1 percent of micro-albuminuria in type 2 diabetes patients without hypertension using 95 percent confidence level and an acceptable margin error of 5 percent and clusters at 1.

Calculation

Total population =240 patients seen in month. Using epiinfo software:

- Go to STATCALC: Then select POPULATION SURVEY
- Expected Frequency of Micro-albuminuria in diabetes is estimated at 12.1 percent in Zambia (Rasmussen et al., 2013).
- Therefore, we used 12.1 as our expected frequency. The acceptable margin of error was 5 percent at 95 percent confidence level. The clusters were kept at 1 and the design effect at 1.0
- These entries gave a total sample size=**97**

3.5 Sampling Techniques

In this study, the sampling of the population was simple random sampling. In this sampling method, each participant has an equal probability of being chosen. It is meant to be an unbiased representation of a group, however, a sampling error can occur if the sample does not end up accurately reflecting the population it is supposed to represent. This could affect the validity and reliability of results.

This was done by selecting every 3rd person out of the 240 total population sample. This was arrived at by dividing the total population, 240 by the sample size, 90 to come up with a consistent way of randomly selecting participants without bias. The target population consisted of type 2 diabetes mellitus patients attending renal clinic at the university teaching hospital. The age group under investigation was between 18 and 72 years. This included a total of 52 female participants and 38 male participants.

3.5.1 Case definition

3.5.2 Type 2 diabetes mellitus

Type 2 diabetes mellitus was defined according to the clinical diagnosis; the study included those already diagnosed clinically as having type 2 diabetes mellitus. This data was obtained from the participants file.

3.5.3 Micro-albuminuria

Micro-albuminuria was diagnosed if the participant had between 30mg- 300mg/24h of urine albumin in spot urine sample collected and Serum creatinine was measured to support the clinical diagnosis. Micro-albuminuria and Serum creatinine values were compared with the baseline values.

3.5.4 Inclusion Criteria

(i) The study included individuals with type 2 diabetes.

(ii) Non-diabetic participants

(iii) Either gender was included.

(iv) Patients who are 18 years and above were recruited.

(v) Participants who voluntarily provided the required written consent after explanation of the study were also included.

3.5.5 Exclusion Criteria

(i) The study excluded all those individuals that failed to provide written consent to the study after explanation was done.

(ii) Pregnant women were also excluded from the study.

(iii) Already established cases of renal failure were excluded.

3.6.0 Data Collection

Eligible type 2 diabetic patients were enrolled in the study. The study was fully explained to the participants and the study commenced after consent had been given. The consent letters were signed by each participant to show consent to the study. Important information like medical history of the patient was collected after signing of the consent form. The demographic data of use was age of the participant as well as sex. Other information that was obtained included fasting blood glucose levels.

3.6.1 Clinical Data

Recruitment of study participants was done on every Tuesday of the week during day time and eligible type 2 diabetes mellitus patients were enrolled to the study. The study was explained to the participants and assigned a serial number upon giving consent.

3.6.2 Specimen Collection

Two (2) ml of blood was collected via vein puncture using the appropriate needle size from the participants recruited. The vein puncture was conducted by qualified medical personnel. The 2ml was then transferred into Lithium heparin (green top) tube.

The container was labeled with the serial number that each participant was assigned at the time of recruitment. The samples were transported to the clinical chemistry laboratory within UTH Immediately. The collected blood was used to measure renal function test (creatinine only). Fresh urine was collected from the participants in sterile urine containers for the determination of micro-albuminuria. The urine samples were also analyzed immediately after collection.

3.6.3 Validity and Reliability

To ensure reliability and validity, calibration and quality control was performed on all the analytical instruments and analyzers to be used for any purpose during specimen analysis according to the UTH quality control guidelines and Sanket laboratory guidelines. Quality control included equipment calibration and analytical control runs on every analysis before each test analysis was conducted.

3.6.4 Type 2 Diabetes mellitus

The fasting blood glucose levels were determined on the day of recruitment and recorded as given by the medical doctors.

3.6.5 Measurement of renal function tests

Renal function test for creatinine was measured using Olympus AU400 chemistry analyzer available in the clinical chemistry laboratory in the UTH. Olympus AU400 is a fully automated, random access chemistry system.

The analytical principle is spectrophotometry and potentiometry and the analytical methods being: Colorimetry, turbidimetry, latex agglutination, homogeneous Immmuno- Assays, indirect ISE (Ion Selective Electrolytes). The results were then interpreted according to the manufacturer's instruction.

3.6.6 Measurement of Micro-albuminuria

Micro-albuminuria was determined using ELISA (Enzyme-linked Immunosorbent Assay) for the in vitro quantitative measurement of human albumin in urine. The 96-microwell plate was coated with polyclonal anti-human albumin antibody for capturing urine micro-albumin. At the beginning of the ELISA, the calibrator and urine sample was pre-diluted a 100-fold with 1 percent BSA-PBS with 0.1 percent Tween 20. Exactly 10ul of pre-diluted calibrator or sample was added to each sample antibody-coated well and incubated for 30 min at 30^{o}C with gentle agitation. After washing 3 times with the washing buffer, 100ul of diluted, HRP-conjugated detecting antibody was added and incubated at 30^{o}C for 1 hour with gentle agitation.

Wells were washed 3 times using the washing buffer and the enzyme substrate, K-blue was added. After incubation for 10 minutes at room temperature, the reaction was stopped by adding 100ul of 1 mol/L of H_2SO_4 and the absorbance at 450nm and 620 nm was read. The results obtained were photocopied and the original copy was kept and whilst the other copy was sent to the patient file so that they can be communicated to the patient by the clinician, during their next appointment for appropriate action where applicable.

3.6 Table 1.0 Summary of variables

The table below is a summary of the variables that were used in this study as well as their description.

VARIABLE	TYPE	SCORE	MEASURE
PREDICTOR VARIABLES:			
1. Diabetes Mellitus	Categorical	Present/Absent	Graphs/Percentage
2. Fasting Blood Glucose	Continuous	High/Low levels	Graphs
3. Hypertension	Categorical	Present/Absent	Graphs/Percentages
4. Sex	Categorical	Male/Female	Graphs/Percentages
5. BMI	Continuous	High/Low levels	Graphs
OUTCOME VARIABLES:			
1. Micro-albuminuria	Continuous	High/low levels	Graphs
2. Serum Creatinine	Continuous	High/low level	Graphs
POTENTIAL CONFOUNDER: AGE	Continuous	Range	Graphs/Percentage

3.8 Analysis

The Data was doubly entered and validated. The entered data was checked for consistency and was then exported to STATA (version 14) for analysis, result summaries were presented in tables and graphs. The comparison of means of micro-albuminuria and serum urea/creatine in type 2 diabetes mellitus and non-diabetic was determined by the student t-test. Pearson's correlation was used to describe the association between continuous variables, micro-albuminuria, serum creatinine, and fasting blood glucose. Tests were interpreted at 5 percent significance level or 95 percent confidence interval. A p-value of <0.05 was taken as indication of statistical significance and validation of finding.

3.9 Ethical considerations

The study was subjected to ethical approval by ERES CONVERGE IRB. IRB number **00005948**. FWA number **00011697**. Approval number: **2017-Dec-002**. Permission to conduct the study was granted by the UTH medical superintendent. A well written consent letter in English and Chinyanja was given to the participants. This explained why the study was conducted, the benefits of the study to the individual and community and also the possible risks likely to be encountered. The participants who gave consent were enrolled.

Venous blood was drawn from consented participants and this caused some discomfort and minimum pain in some participants. Vein puncture was performed via standard techniques and skin preparation (with alcohol), by qualified medical personnel to minimize the risks of developing a hematoma or infection at the site of puncture. Urine for the purpose of the study was also obtained from the participants and this did not cause discomfort as it was through normal urination. Data was handled in a confidential manner to prevent loss of privacy. Paper files were kept in a locked cabinet in a secure laboratory. Electronic files were stored in an encrypted and password protected database on secure laptop. Only the primary investigator and members of the research team who contact the subjects have access to information linked to subject identifiers. Participants with higher than normal levels of micro-albuminuria were recommended for further tests and possible treatment in order to manage the condition.

Summary

This chapter explored the methods used in this research. It clearly explained the techniques used in sampling and also setting up of the study. This section also mentioned the inclusion and exclusion criteria. The procedures used to carry out each particular stage of this research have been clearly explained in this section. The collection of data is also outlined as well as the analysis of the data that was collected. This included the analytical biochemistry techniques applied for each of the experiments conducted. The analysis of blood serum and also that of micro-albuminuria was clearly explained. The statistical tool used for analysis of data was also mentioned and the technical inputs to be generated and the nature of the software.

CHAPTER FOUR

PRESENTATION OF FINDINGS

Overview

This section presents the findings of this study. It shows the characteristics of the participants that were enrolled in this study. The groups included in the study and how the outcomes of the experiments conducted. It also explains the observations in the findings in this study. This chapter gives information on the values obtained in experiment. It shows the levels of micro-albuminuria and serum creatinine in diabetes mellitus patients and also non-diabetic participants.

4.1. Characteristics of participants

The study enrolled a total of 90 participants. This study group includes 45 non-diabetic controls and 45 type 2 diabetes mellitus patients attending renal clinic at University Teaching Hospital, in Lusaka, Zambia. A total of 28, (62.2 percent) of diabetes mellitus patients had micro-albuminuria and 17, (38.8 percent) had no micro-albuminuria. In the non-diabetic control group, 8, (17.8 percent) of the participants had micro-albuminuria while 37, (82.2 percent) had no micro-albuminuria. The total of female participants was 52, (57.8 percent) and male participants were 38, (42.2 percent) respectively.

The total number of hypertensive participants was 47, (52.2 percent) with 26, (28.9 percent) being diabetes mellitus patients and 21, (23.3 percent) were non-diabetic controls. The age range was from 18years to 72 years of age. The mean age of the participants was estimated at 52years and the standard deviation was 13.63917. The mean and standard deviation for micro-albuminuria were 34.542 mg/dl and 21.11558 respectively. Log of Micro-albuminuria mean (3.343835mg/dl) and standard deviation (0.5703127). The mean and standard deviation for serum creatinine levels were 71.25889 umol/L and 28.6387 respectively. Log of Serum Creatinine mean (4.194105umol/L) and standard deviation, (0.3798605). Fasting blood glucose mean, (6.525556)mmol/L and standard deviation, (1.444444.) Table 1.0 shows a summary of the descriptive statistics.

4.2 Table 1.0. Summary of descriptive statistics

CATEGORICAL VARIABLES	**FREQUENCY**	**PERCENTAGE**
Diabetics	45	50
Non diabetics	45	50
Hypertension	47	52.2
Normotensive	43	47.8
Female	52	57.8
Male	38	42.2
CONTINUOUS VARIABLES	MEAN	SD
Log10 Micro-albuminuria (mg/dl)	3.343835	0.5703127
Log10 Serum Creatinine (umol/L)	4.194105	0.3798605
Fasting Blood Glucose(mmol/L)	6.525556	1.444444
Age, (years)	51.53333	13.63917
Micro-albuminuria (mg/dl)	34.542	21.11558
Serum Creatinine (umol/L)	71.25889	28.6387

4.3. Mean Micro-albuminuria and Serum Creatinine Levels in diabetes mellitus and non-diabetic participants.

The mean micro-albuminuria levels in type 2 diabetes mellitus patients was, **40.9971 mg/dl** compared to **26.0869mg/dl** in non-diabetic controls. The blue bars in **fig.1** show the mean micro-albuminuria levels in type 2 diabetes mellitus patients and non-diabetic controls. Fig.1 shows higher values of micro-albuminuria levels in type 2 diabetes mellitus patients compared to non-diabetic controls. This difference in mean values was statistically significant, ***p*=0.0001**.

The mean serum creatinine levels in type 2 diabetes mellitus and non-diabetic controls is represented by maroon bars fig.1. The mean serum creatinine levels in type 2 diabetes mellitus patients was **80.9711(umol/L)** compared to **61.5467(umol/L**) in non-diabetic controls. This indicates a higher serum creatinine level in type 2 diabetes mellitus patients compared to non-diabetic participants. There was a statistically significant difference in the mean serum creatinine levels between type 2 diabetes mellitus patients and the non-diabetic controls, ***p*= 0.0003**.

Fig. 1 shows a summary of the mean differences in micro-albuminuria and serum creatinine levels in type 2 diabetes mellitus patients and non-diabetic controls. The mean micro-albuminuria and serum levels were compared using student t-test after log transforming the data to ensure it assumes normal distribution.

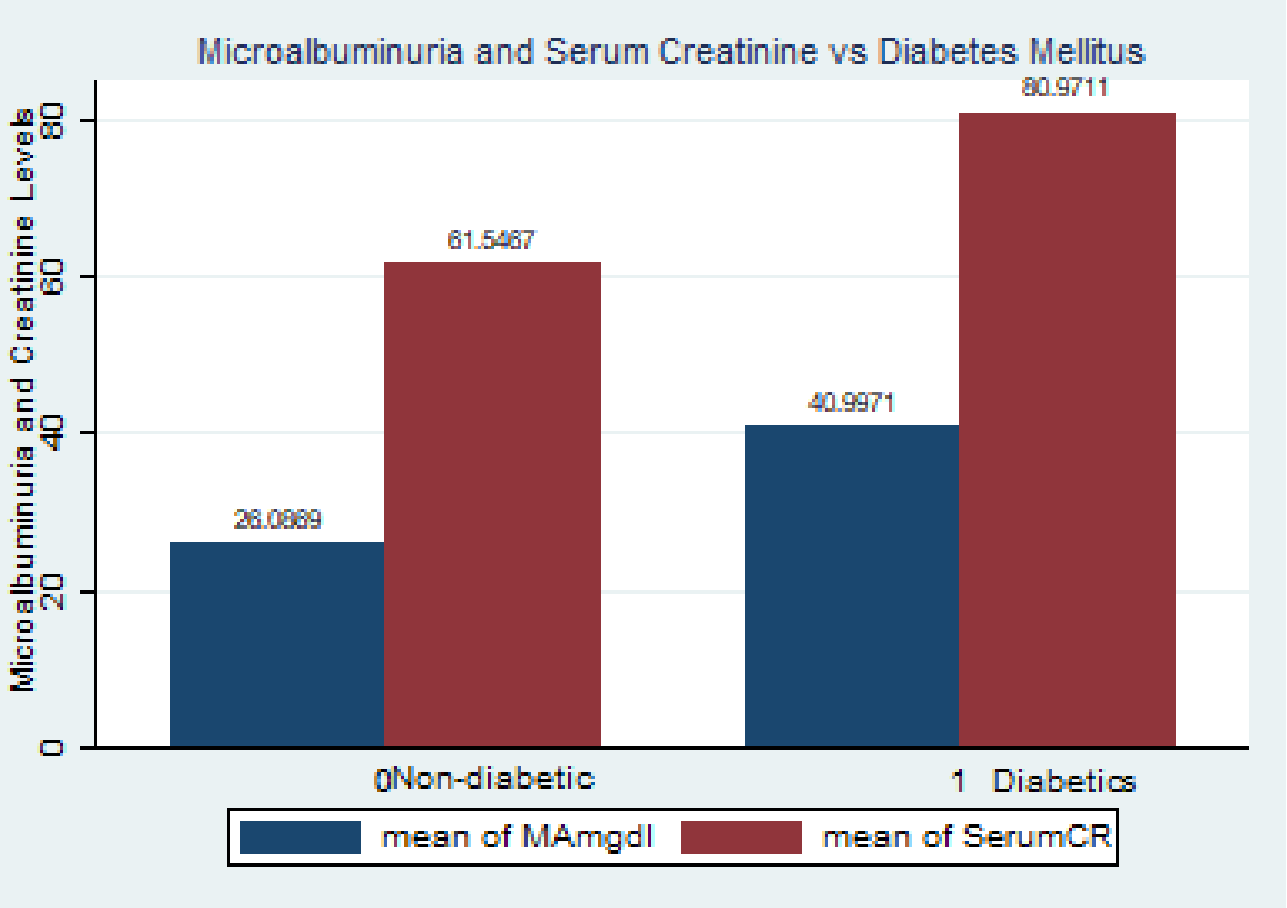

Figure 1: Mean Microalbuminuria and serum creatinine levels in type 2diabetes mellitus and non-diabetic controls. *p*=0.0001 for micro-albuminuria and *p*=0.0003 for serum creatinine.

4.4. Mean Micro-albuminuria and Serum Creatinine levels in Type 2 Diabetes Mellitus and Hypertension.

In fig.2, the blue bars represent the mean micro-albuminuria levels while the orange-red bars represent serum creatinine levels in type 2 diabetes and hypertension. The non-diabetic controls without hypertension showed the lower mean of micro-albuminuria, **(19.1663 mg/dl)** compared to **(27.4874 mg/dl)** in type 2 diabetes mellitus without hypertension.

A mean micro-albuminuria level of **33.9962mg/dl** was observed in non-diabetic but hypertensive individuals compared to **50.8696mg/dl** in type 2 diabetes mellitus with hypertension. This showed that non-diabetic controls without hypertension had the lowest mean levels of micro-albuminuria and type 2 diabetes mellitus patients with hypertension had the highest mean micro-albuminuria levels, ***p*<0.0001**. The mean serum creatinine levels in non-diabetic controls without hypertension was **50.3083umol/L** compared to **64.3053umol/L** in type 2 diabetes mellitus patients without hypertension.

Non-diabetic controls with hypertension had a mean of **74.3905umol/L** compared **to 93.15umol/L** in type 2 diabetes mellitus with hypertension. This also shows that participants with both diabetes mellitus and hypertension had higher mean levels of serum creatinine compared to diabetes mellitus only, hypertension only or the controls without both diabetes mellitus and hypertension, **p<0.0001**.

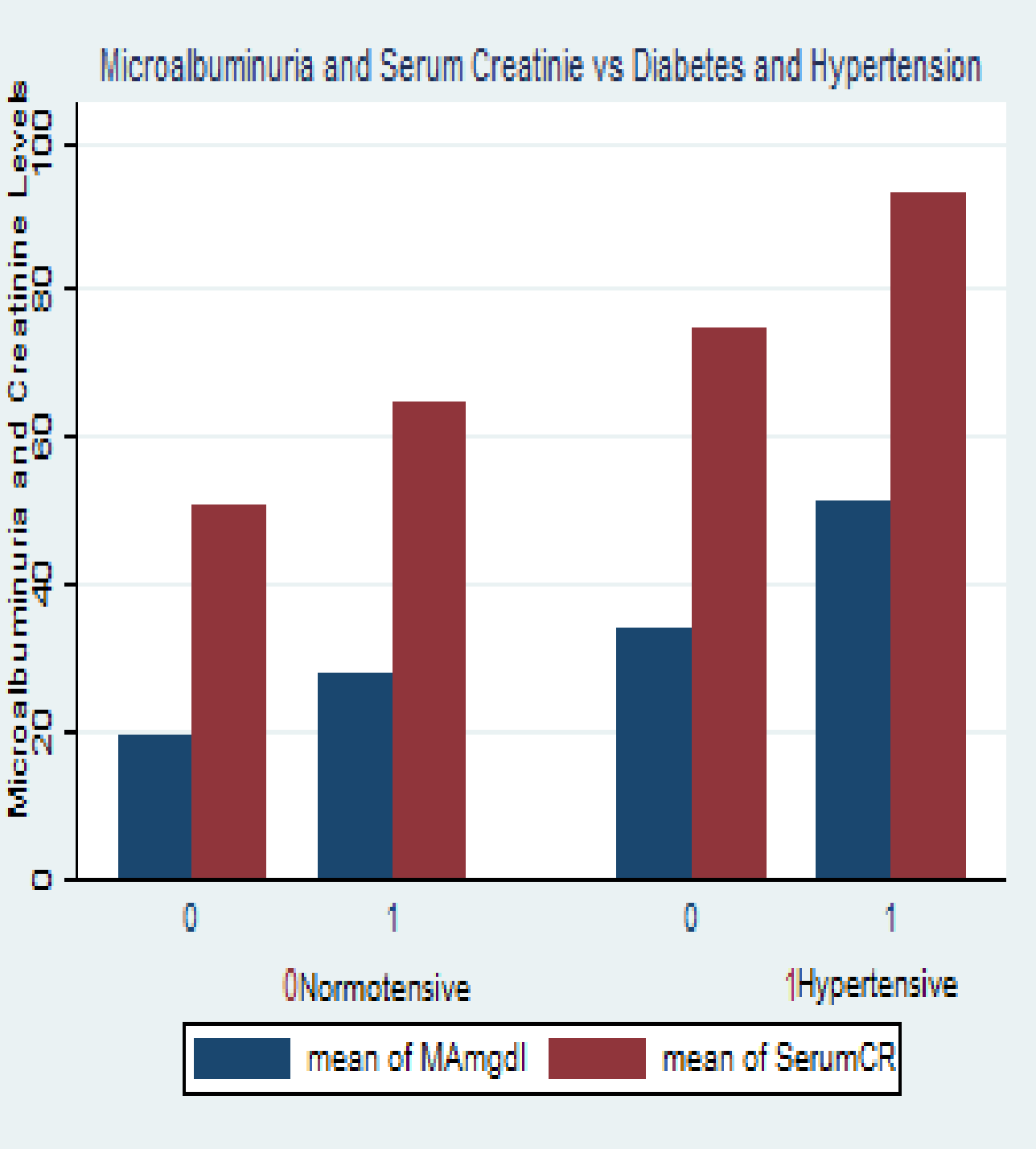

Figure 2: Mean Microalbuminuria and Serum Creatinine levels in type 2 diabetes mellitus and hypertension.

4.5. **Pearson's correlation of Serum Creatinine and Fasting Blood Glucose in type 2 diabetes mellitus and non-diabetic controls.**

Pearson's correlation analysis showed a positive linear relationship between serum creatinine levels and fasting blood glucose levels in type 2 diabetes mellitus. Fig.3 shows that higher levels of serum creatinine positively correlated with higher fasting glucose levels. There was a weak positive correlation between serum creatinine and fasting blood glucose levels, ***r* =0.2995 and p=0.004** showing a statistical significance. The serum creatinine levels increased with an increase in fasting blood glucose levels. Fig.3 shows the linear relationship between serum creatinine levels and fasting blood glucose levels.

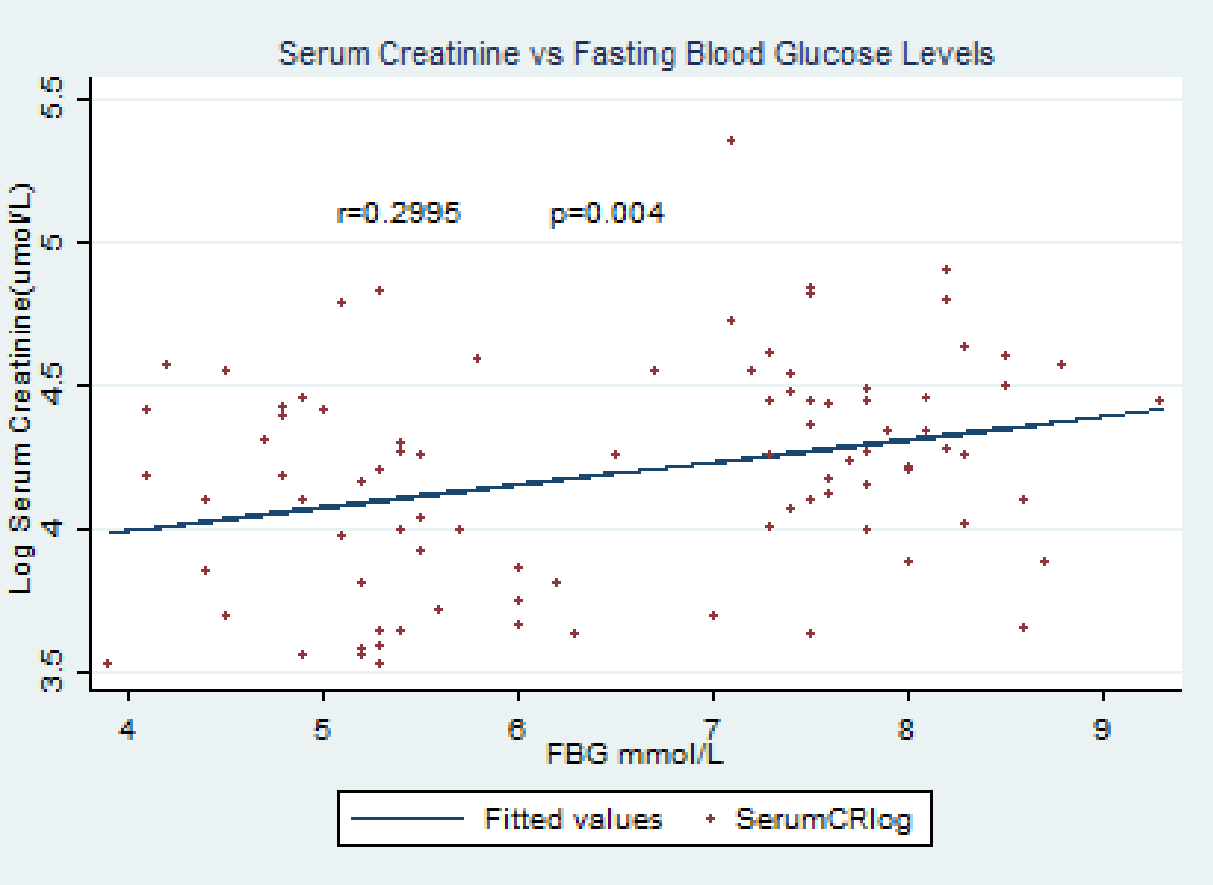

Fig 3: Pearson's correlation of Serum Creatinine and Fasting Blood Glucose in type 2 diabetes mellitus and non-diabetic controls.

4.6. Pearson's correlation analysis of Microalbuminuria and Fasting Blood Glucose Levels in Type 2 Diabetes Mellitus and Non-Diabetic controls.

The Pearson's correlation analysis between micro-albuminuria and fasting blood glucose showed a positive correlation in type 2 diabetes mellitus and non-diabetic controls. Micro-albuminuria increased with an increase in fasting blood glucose levels indicating higher levels of micro-albuminuria in type 2 diabetes mellitus patients, than non-diabetic controls. Fig. 4 shows a weak positive linear relationship between micro-albuminuria and fasting blood glucose levels was seen in type 2 diabetes mellitus and non-diabetic controls, ***r*=0.3092 and *p*=0.0030.**

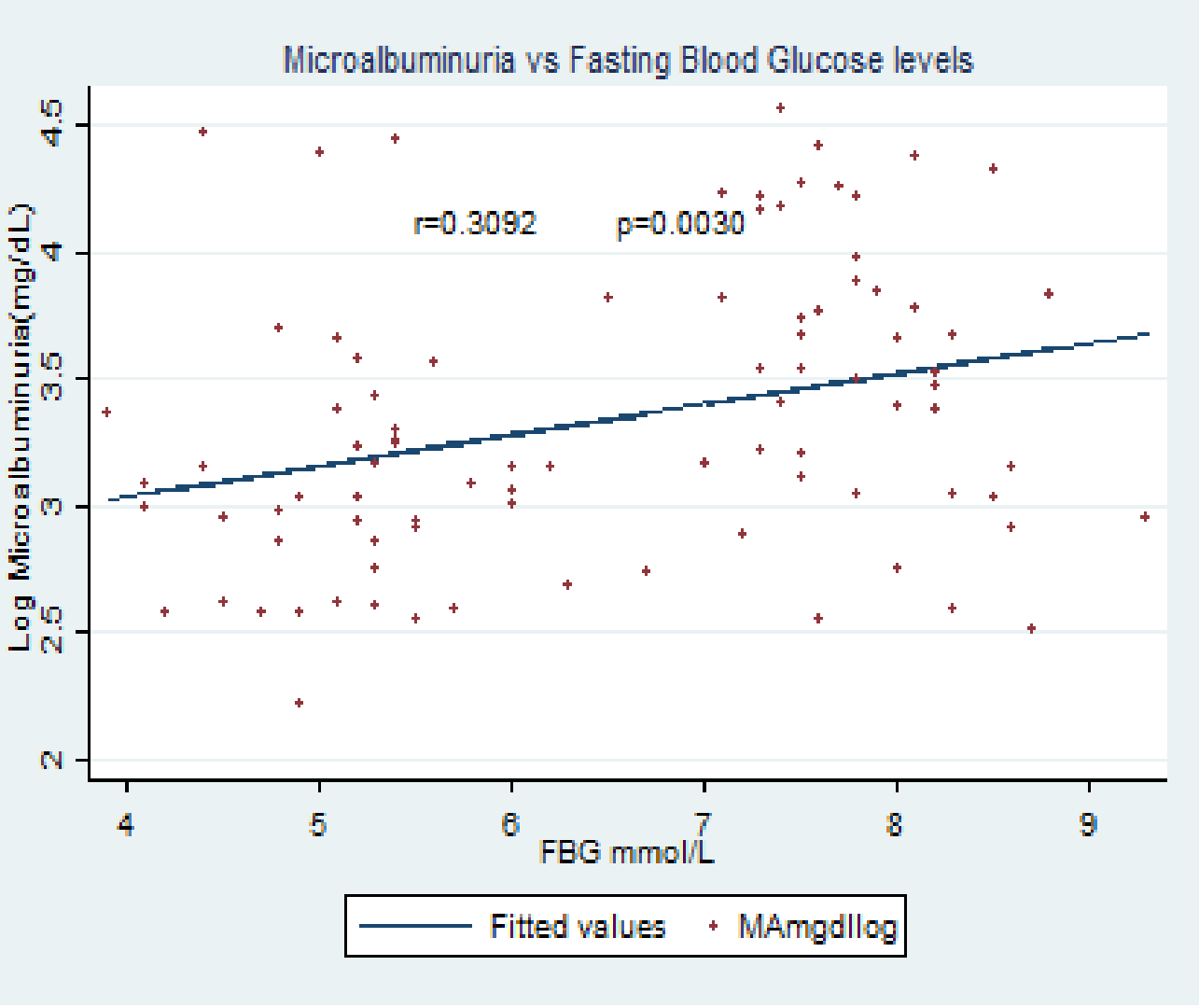

Fig 4: Pearson's correlation analysis of Microalbuminuria and Fasting Blood Glucose Levels in Type 2 Diabetes Mellitus and Non-Diabetic controls.

4.7. **Pearson's correlation analysis between Microalbuminria and Serum Creatinine Levels in type 2 diabetes mellitus and non-diabetic controls.**

The Pearson's correlation analysis between micro-albuminuria and Serum Creatinine in diabetic and non-diabetic participants showed a weak positive correlation ***r*=0.3475** and ***p*=0.008.** Micro-albuminuria increased with an increase in Serum Creatinine levels in diabetic and non-diabetic participants. Fig.5 shows a weak positive linear relationship between micro-albuminuria levels and serum creatinine levels in type 2 diabetes mellitus patients and non-diabetic controls.

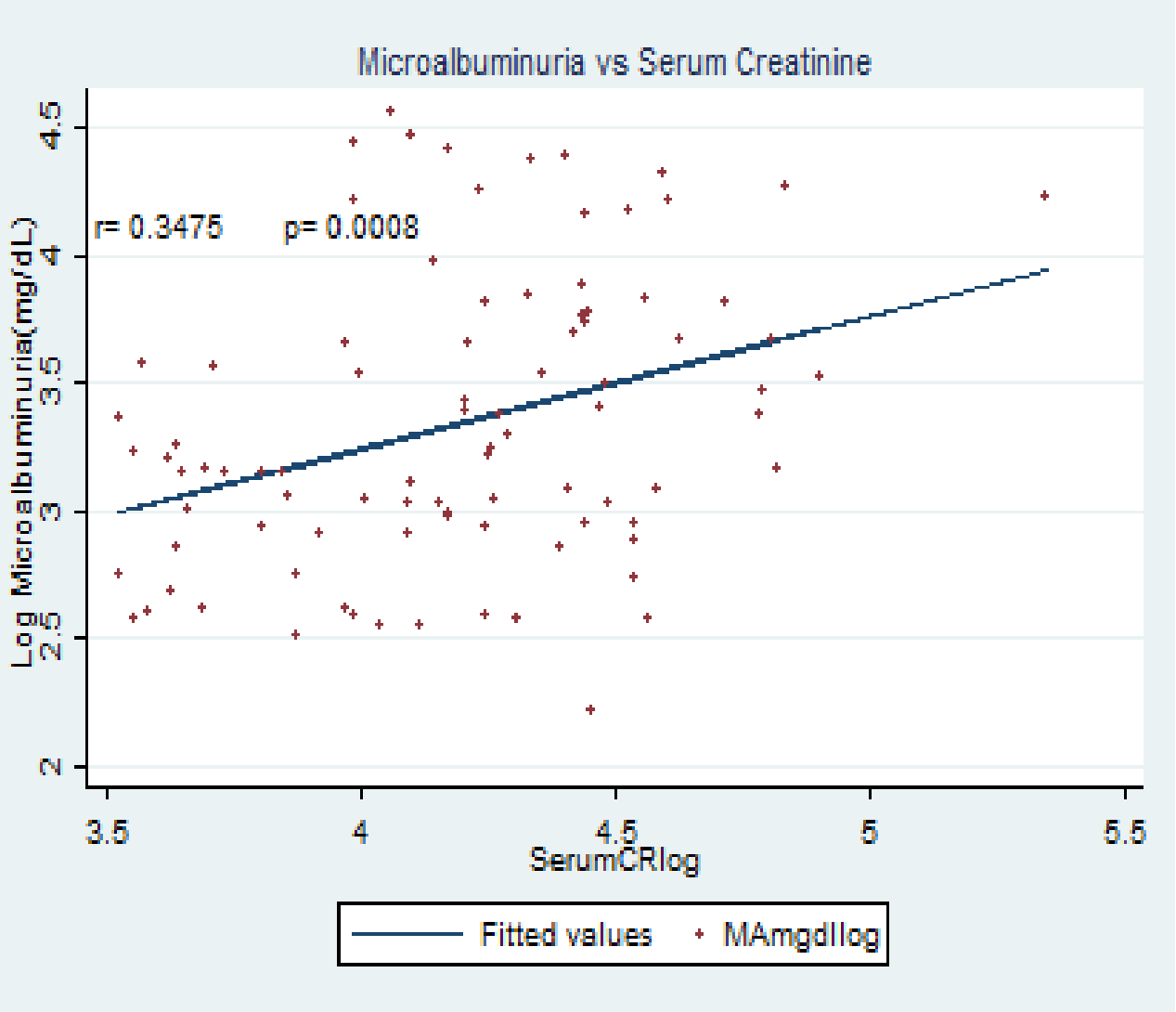

Fig 5: Pearson's correlation analysis between Micro-albuminria and Serum Creatinine Levels in type 2 diabetes mellitus and non-diabetic control

CHAPTER FIVE

DISCUSSION OF FINDINGS

Overview

This section gives a critical analysis of the findings of the study. It also gives a comparative analysis of what other researchers have done. Any differences or similarities with other research findings will be outlined in this section. Scientific explanations to the observed outcomes will also be given.

5.1 Levels of Micro-albuminuria in Type 2 diabetes mellitus and non- diabetic participants

In this study, the mean micro-albuminuria and serum creatinine levels were determined in non-diabetic controls and type 2 diabetes mellitus patients attending renal clinic at University Teaching Hospital, in Lusaka, Zambia. Furthermore, the correlation between micro-albuminuria and serum creatinine levels in type 2 diabetes mellitus was established. From the results, it is evident that type 2 diabetes mellitus patients have higher mean values of micro-albuminuria compared to non-diabetic controls as shown by the values in fig. 1. There was a statistically significant difference in the mean micro-albuminuria levels between type 2 diabetes patients and non-diabetic controls, $\boldsymbol{p=0.0001}$. This observation clearly indicates that individuals with diabetes mellitus have higher levels of micro-albuminuria. Type 2 diabetes mellitus patients in this study showed consistently high levels of micro-albuminuria compared to the non-diabetic participants.

From other studies, it has been shown that there is a high prevalence of micro-albuminuria in type 2 diabetic outpatients (Abougalambou and Abougalambou, 2013). This simply implies that individuals with type 2 diabetes mellitus are more likely to develop micro-albuminuria and eventually have higher levels with time.

It is justified by further research showing that micro-albuminuria levels are significantly associated with diabetes mellitus (Chuengsamarn et al., 2014). With these findings, it is crucial to seek tight screening measures for micro-albuminuria in individuals newly diagnosed with type 2 diabetes mellitus. It must be noted that, micro-albuminuria is said to be associated with cardiovascular risk even in pre-diabetes and prehypertension (Sriharibabu et al., 2014).

Hence, there is need for effective methods to slow down the progression to complications of diabetes mellitus. This observation can be supported by scientific evidence indicating that diabetes mellitus could have long lasting effects on the ability of the kidney to filter out substances. Type 2 diabetes mellitus patients with micro-albuminuria have a huge decline in glomerular filtration rate (GFR) compared to normo-albuminuric type 2 diabetic subjects (Eboh and Chowdhury, 2015). It is this decline in glomerular filtration rate that leads to leakage of the protein albumin in urine hence bringing about complications. Prolonged hyperglycemia can lead to glomerulosclerosis, which is the hardening of the glomeruli in the kidney. It is a general term used to describe scarring of the kidneys' tiny blood vessels, the glomeruli, found in the kidney that filter off substances from the blood. Therefore, micro-albuminuria is one of the signs of glomerulosclerosis. This damage caused by diabetes mellitus disturbs the filtering process of the kidneys and allows protein to leak from the blood into urine.

On the other hand, a certain study concluded that diabetes mellitus is the likely cause of albuminuria in patients with persistent micro-albuminuria or macro-albuminuria who have had diabetes for at least 10 years and/or diabetic retinopathy (Satirapoj and Adler, 2015). The duration of diabetes also has a role to play on the integrity of the glomerular filtration barrier. The long the duration of diabetes, the more compromised the structure of the filtration barrier becomes hence allowing large protein molecules like albumin to pass into urine.

Interestingly, in all these findings, there could be an indication that the risk of micro-albuminuria increases with poor glycemic control (Idogun and Kasia, 2011). This therefore demands that people with diabetes mellitus should thrive to maintain glycemic control to enable them maintain the glomerular filtration barrier. It also implies that the duration of diabetes can have less impact if the patient is able to keep the blood glucose levels in check and avoid spikes in the levels from time to time. It is important to monitor how the sugar levels fluctuate within the body of a patient and give suggestions on how the fluctuations can be reduced.

5.2 Serum Creatinine Levels in type 2 diabetes mellitus and non-diabetic participants

It was observed in this research that, there were higher levels of serum creatinine in type 2 diabetes mellitus compared to non-diabetic controls. The presence of high levels of serum creatinine could be a result of so many different processes in the body. The breakdown of creatine leads to the production of creatinine in body. However, if the kidney is functioning properly, the levels of serum cretainine are supposed to be well regulated as this waste product needs to be eliminated from the blood stream. The observation made shows that patients with type 2 diabetes mellitus have compromised kidney function as evidenced by the high levels of serum creatinine. Interestingly, some studies show that patients with diabetes more than six years' duration have higher serum urea and serum creatinine levels compared to diabetics of lesser duration (Bamanikar et al., 2016). The duration of diabetes seems to have a clear impact on renal function. Within the diabetes mellitus group, the levels of serum creatinine may also vary according to the duration of diabetes. This means that those patients who have been diabetic for a longer period of time have higher levels serum creatinine, as concluded in the study mentioned above.

However, the results in this study show a significant statistical difference in serum creatinine levels between type 2 diabetes mellitus patients and non-diabetic controls, ***p*=0.0003**. These findings are consistent with other recent findings that have so far established a statistically significant difference in mean serum creatinine levels between diabetic groups and non-diabetic controls (Chutani and Pande, 2017). However, some studies show that the mean serum creatinine levels in diabetic groups are higher than non-diabetic groups but the difference is not statistically significant (Kumar et al., 2015). Surprisingly, the significance of the levels of creatinine in diabetes mellitus patients is very debatable with some studies showing a statistical difference and yet others showing no statistical difference.

A few more studies have not found any significant difference in the mean serum creatinine levels between diabetic groups and non-diabetic controls (Bamanikar et al., 2016).

The reason for this disparity could be due to difference in the duration of diabetes in the enrolled participants. The studies that do not show a statistically significant difference in mean serum creatinine levels between type 2 diabetes mellitus patients could have enrolled newly diagnosed diabetes patients whose kidney function is not compromised. These newly diagnosed diabetic patients may not have similar kidney function compared to already established cases that have lived many years with diabetes. This could be because, in patients with type 2 diabetes, age or age at diagnosis and diabetes duration are independently associated with macro-vascular events that could lead to kidney damage (Zoungas et al., 2014). The damage to the kidney usually occurs gradually and becomes worse with time. Another reason could be due to different amounts of creatinine produced by the body from one individual to another. Since creatinine is a waste product from muscle breakdown, those with more tear and wear of muscles could have higher levels of creatinine in the blood stream.

5.3 Hypertension and Type 2 diabetes mellitus

In this study, the combination of both type 2 diabetes mellitus and hypertension showed a higher mean compared to diabetes only, hypertension only, and non-diabetic controls without hypertension. Primary hypertension and type 2 diabetes mellitus are the major causes of renal damage and cardiovascular events. The co-existence of these conditions further increase the risk of progressive renal disease, cardiovascular events and mortality. Urinary excretion of albumin (micro-albuminuria) and lowered GFR are the early markers of such tendency (Saha et al., 2015). This is definitely a deadly combination in that both diabetes mellitus and hypertension have serious adverse effects on the integrity of the glomerular filtration barrier. Hypertension also increases damage on the blood vessels that cannot withstand the high pressure at which blood is flowing, hence hypertensive patients with type 2 diabetes mellitus present micro-albuminuria more frequently (Gluhovschi et al., 2016). This will eventually lead to the acceleration and the progressive increase in Micro-albuminuria and decline in glomerular filtration (Marques da Silva et al., 2015).

Many studies suggest that anti-hypertensive therapy to micro-albuminuric diabetic patients with hypertension can reduce albumin excretion rate (AER) and rate of decline of GFR. In type 2 diabetes, treatment of micro-albuminuria with angiotensin converting enzyme inhibitors (ACEI) can reduce AER and rate of decline of GFR significantly more than calcium antagonists (Eboh and Chowdhury, 2015). Glomerulosclerosis caused by diabetes mellitus and also the high blood pressure due to hypertension will lead to rapid deteriorating of the structure of the kidney.

This could be the main reason why participants with both diabetes mellitus and hypertension showed higher levels of micro-albuminuria and serum creatinine compared to non-diabetic controls enrolled in this study.

5.4 The correlation between Micro-albuminuria Serum creatinine levels

The pearson's correlation analysis that was conducted in this study shows a weak positive relationship between micro-albuminuria, serum creatinine levels and fasting blood glucose levels. Increase in levels of plasma creatinine, urine micro-albuminuria was observed among type 2 diabetes mellitus patients. The results also show a weak positive correlation between micro-albuminuria and serum creatinine levels in type 2 diabetes mellitus patients, **r=0.3475** and ***p*=0.0008**. In one study, micro-albuminuria was seen to increase with an increase in serum creatinine and a positive correlation was observed between serum creatinine levels and micro-albuminuria in diabetes and hypertension as compared to control group (Keshab et al., 2013).

5.5 Correlation between Micro-albuminuria and fasting blood glucose levels

There was also a weak positive correlation between micro-albuminuria and fasting glucose levels, **r =0.3092 and *p*=0.0030.** These observations indicate that intensive glucose control starting at the time of diagnosis is associated with a significantly decreased risk of all major vascular complications and mortality (Satirapoj and Adler, 2015).This means an increase in fasting blood glucose levels would result in an increase in micro-albuminuria. This is also consistent with other studies that found a moderate positive correlation between micro-albuminuria and plasma creatinine (Karar et al., 2015).

In type 2 diabetes mellitus patients, and non-diabetic controls, levels of serum creatinine will increase with an increase in micro-albuminuria.

This clearly indicates that type 2 diabetes mellitus patients need early screening for the presence of micro-albuminuria. The results have clearly shown that diabetes mellitus could lead to many serious complications if not managed well. Type 2 diabetes patients have shown significant levels of micro-albuminuria, serum creatinine as well as fasting blood glucose levels. A combination of all these conditions makes type 2 diabetes mellitus a life threatening disease. In patients with diabetes, the presentation of a rapidly rising urinary protein level, a more rapid loss of renal function ($\geq$ 1 ml/min/month), active urine red cell or white cell casts, gross hematuria, systemic signs and/or symptoms of other glomerular diseases, known chronic infections such as HIV or hepatitis B or C, and/or renal impairment without diabetic retinopathy should lead to the consideration of renal biopsy (Satirapoj and Adler, 2015).

Summary

This section presented the findings of this study. It showed the characteristics of the participants that were enrolled in this study. The groups included in the study and how the outcomes of the experiments conducted. It also explained the observations in the findings in this study. It gave a detailed discussion of the main reasons for the similarities and disparities observed in this study with reference to other studies.

CHAPTER SIX
CONCLUSION AND RECOMMENDATIONS

Overview

This section offers a conclusion and recommendations of the study. It clearly outlines what conclusions can be drawn from the observation made in the findings. The future recommendations have been that can help improve the on clinical outcomes of patients living with diabetes mellitus.

5.1. Conclusion

In Zambian setting, both serum creatinine and micro-albuminuria levels are significantly higher in type 2 diabetes patients compared to controls. Fasting blood glucose and Serum creatinine all correlated with micro-albuminuria. Further studies to explain the mechanism of micro-albuminuria in type 2 diabetes in a Zambian setting would be very important. The presence of Micro-albuminuria is an indication requiring many steps to prevent further renal damage by correction of risk factors, such as control of diabetes and hypertension. The findings call for more aggressive screening and intervention of micro-albuminuria in diabetic patients.

5.2. Recommendations

Modern technological advances are fast growing and there is need for more accurate methods of early detection of Micro-albuminuria in type 2 diabetes mellitus. To avoid progression to diabetic nephropathy, it is very important to devise strict screening routines for newly diagnosed type 2 diabetes mellitus patients. Current studies are slowly demonstrating that the current cut off point used to determine micro-albuminuria may be late. This then calls for urgent research in our country to establish which markers could help in early detection to avoid catching the diseases a bit too late. Furthermore, the current use of fasting blood glucose is slowly being challenged by the latest model, the continuous glucose monitoring (CGM) technology which is being used to evaluate how blood glucose fluctuates in individuals over time and not just using the concentration taken on one reading.

Summary

This section offered a conclusive analysis of findings and recommendations of the study. It clearly outlined what conclusions can be drawn from the observation made in the findings. The future recommendations have been given that can help improve the clinical outcomes of patients living with diabetes mellitus.

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APPENDICES

1.0 Permission to conduct research at UTH

Shandele Ginnethon Chaamba

C/O University Of Zambia

School Of Medicine

P.O.BOX 50110

Lusaka

22.11.2017

The Director,

Health Clinical Care

University Teaching Hospital,

Lusaka,

Zambia.

Dear Sir/Madam

RE: **PERMISSION TO CONDUCT A RESEARCH AT THE RENAL CLINIC, UTH.**

Reference is made to the above captioned. My name is Shandele Ginnethon Chaamba. I am a postgraduate student in the School of Medicine pursuing my MSc-Biochemistry. I successfully completed my one year of course work and would love to embark on my research.

It is for this reason that I write to seek permission to conduct my research at University Teaching Hospital, Renal clinic. My data collection is scheduled to commence immediately after ethical approval is granted. Your permission will enable me apply for ethical approval.

Find attached the summary of my research proposal. Your assistance in this matter will be greatly appreciated.

Yours Faithfully,

Shandele Ginnethon Chaamba

MSc-Biochemistry Student.

Email:shandeleginnethon@yahoo.com

Cell: 0978602224

REPUBLIC OF ZAMBIA
HEAD
CLINICAL CARE
28 NOV 2017
UNIVERSITY TEACHING HOSPITAL
P/BAG RW 1X, LUSAKA
28.11.2017

2.0. Ethical Approval

ERES CONVERGE

+260 955 155 634
Cell: +260 966 765 503
Email: eresconverge@yahoo.co.uk

I.R.B. No. 00005948
F.W.A. No. 00011697

7th February, 2018

Ref. No. 2017-Dec-002

The Principal Investigator
Mr. Shandele G. Chaamba
The University of Zambia
School of Medicine
Dept. of Physiological Sciences
P.O. Box 50110,
LUSAKA.

Dear Mr. Chaamba,

RE: MICROALBUMINURIA AS A PREDICTOR OF DIABETIC NEPHROPATHY IN DIABETES MELLITUS PATIENTS ATTENDING RENAL CLINIC AT THE UNIVERSITY TEACHING HOSPITAL, LUSAKA ZAMBIA

Reference is made to your corrections and subsequent meeting. The IRB resolved to approve this study and your participation as principal investigator for a period of one year.

Review Type	**Ordinary**	Approval No. **2017-Dec-002**
Approval and Expiry Date	Approval Date: 7th February, 2018	Expiry Date: 6th February, 2019
Protocol Version and Date	Version-Nil	6th February, 2019
Information Sheet, Consent Forms and Dates	• English, Nyanja.	6th February, 2019
Consent form ID and Date	Version-Nil	6th February, 2019
Recruitment Materials	Nil	6th February, 2019
Other Study Documents		6th February, 2019
Number of participants approved for study	170	6th February, 2019

Specific conditions will apply to this approval. As Principal Investigator it is your responsibility to ensure that the contents of this letter are adhered to. If these are not adhered to, the approval may be suspended. Should the study be suspended, study sponsors and other regulatory authorities will be informed.

Conditions of Approval

- No participant may be involved in any study procedure prior to the study approval or after the expiration date.
- All unanticipated or Serious Adverse Events (SAEs) must be reported to the IRB within 5 days.
- All protocol modifications must be IRB approved prior to implementation unless they are intended to reduce risk (but must still be reported for approval). Modifications will include any change of investigator/s or site address.
- All protocol deviations must be reported to the IRB within 5 working days.
- All recruitment materials must be approved by the IRB prior to being used.
- Principal investigators are responsible for initiating Continuing Review proceedings. Documents must be received by the IRB at least 30 days before the expiry date. This is for the purpose of facilitating the review process. Any documents received less than 30 days before expiry will be labelled "late submissions" and will incur a penalty.
- Every 6 (six) months a progress report form supplied by ERES IRB must be filled in and submitted to us.
- ERES Converge IRB does not "stamp" approval letters, consent forms or study documents unless requested for in writing. This is because the approval letter clearly indicates the documents approved by the IRB as well as other elements and conditions of approval.

Should you have any questions regarding anything indicated in this letter, please do not hesitate to get in touch with us at the above indicated address.

On behalf of ERES Converge IRB, we would like to wish you all the success as you carry out your study.

Yours faithfully,
ERES CONVERGE IRB

Prof. E. Munalula-Nkandu
BSc (Hons), MSc, MA Bioethics, PgD R/Ethics, PhD
CHAIRPERSON