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MOI UNIVERSITY

SCHOOL OF MEDICINE

Persistent Albuminuria in Patients Newly Diagnosed with Type 2 Diabetes Mellitus at Moi Teaching and Referral Hospital, Eldoret, Kenya.

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SM/PGM/01/11

A Thesis Submitted to the School of Medicine, College of Health Sciences in Partial Fulfillment of the Requirement for an Award of the Degree of Master of Medicine in Internal Medicine of Moi University

DECLARATION

Declaration by Candidate

This thesis is my original work and has not been presented for an award of a degree or an academic credit in any university. No part of this thesis may be reproduced without the permission of the author and/or Moi University.

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DEDICATION

This thesis is dedicated to my husband Gilbert for his love and support, my son Kim for he is the gift of joy in my life.

I also dedicate this to my family; my mum Jane who is my inspiration, my siblings Alice, Janet, Emily, Stanley, Caro, Nick, Gladys and Lorna for their love and care**.**

Title: Persistent albuminuria in patients newly diagnosed with type 2 diabetes mellitus at Moi Teaching and Referral Hospital, Eldoret, Kenya.

ABSTRACT

Background: Persistent albuminuria is a marker of early diabetic nephropathy and increased cardiovascular morbidity and mortality. Therefore, there is need for screening for persistent albuminuria to prevent these complications. While early intervention could retard the progression of nephropathy in patients with persistent albuminuria, screening is not routinely done especially in resource limited settings and even the prevalence of persistent albuminuria is not widely known.

Objectives: To determine the prevalence and associated clinical characteristics of persistent albuminuria among patients newly diagnosed with type 2 diabetes mellitus at MTRH.

Methods: A cross sectional study on patients newly diagnosed with type 2 DM was conducted at the diabetic and medical outpatients' clinics of Moi Teaching & Referral Hospital (MTRH), western Kenya. All patients who met the inclusion criteria were enrolled by consecutive sampling until the desired sample size was obtained. Data was collected using a structured interviewer administered questionnaire. Blood samples for fasting lipid profile, fasting blood sugar, serum creatinine and HbA1c, as well as a random urine sample for Urine-Albumin-Creatinine-Ratio (UACR) were obtained. Those who had UACR above 30mg/24hr had a repeat test done 2-3 months apart. Data was entered into Microsoft access database and analyzed using STATA where descriptive statistics were summarized in tables and graphs.

Results: A total of 205 patients were screened between January and December 2013, with 92 patients (58% female) being enrolled into the study. Mean age was 55±12.5 years, mean BMI was 26.9±4.9 Kg/m^2 and median duration of illness was 9 (IQR 3-14) months. Nearly half of the patients, 44 (47.8%) were known hypertensive, while 4 (4.4%) reported previous stroke. Few patients, 8 (8.7%) were on statins and aspirin 6 (6.5%). At enrollment, hypertension was recorded among 26 (28.25%) with the majority, 24 (55.5%) being known hypertensive patients. A third of the patients, 30 (32.6%) were on either angiotensin converting enzyme inhibitors or angiotensin receptor blockers. The majority of patients, 66 (71.7%) were on oral hypoglycemic agents, 22 (23.9%) on insulin alone, 3 (3.3%) on both oral hypoglycemic agents and insulin, while 1 (1.1%) patient was on diet alone. Most patients had poor glycemic control with 16 (17.4%) patients achieving a target HbA1c of ≤7%. Dyslipidemia was prevalent among 88 (96%) patients, with majority 76 (82.6%) having low High Density Lipoprotein (HDL) and 57 (62%) had high Low Density Lipoprotein (LDL). At the initial screening, 27 (29.4%) of the patients had spot albuminuria and 17 (18.5%) had persistent albuminuria. Over 90% of patients had estimated Glomerular Filtration Rate (eGFR) of >60. There was a positive association between, high serum creatinine (P=0.029), low eGFR (P=0.016) and persistent albuminuria while no associations were found with other clinical characteristics.

Conclusion: There is a high prevalence of persistent albuminuria in patients newly diagnosed with type 2 DM. Although known risk factors for albuminuria such as hyperglycemia, dyslipidemia and hypertension were prevalent, only a positive association between high serum creatinine, low eGFR and persistent albuminuria was found.

Recommendation: Screening for persistent albuminuria, dyslipidemia and serum creatinine should be done at diagnosis of all patients with type 2 DM.

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LIST OF ABBREVIATIONS

ACEI	Angiotensin Converting Enzyme Inhibitor
ADA	American Diabetic Association
ARB	Angiotensin Receptor Blocker
BMI	Body Mass Index
BP	Blood Pressure
DM	Diabetes Mellitus
ESRD	End-Stage Renal Disease
FBS	Fasting Blood Sugar
HbA1c	Glycosylated Haemoglobin
IREC	Institutional Research and Ethics Committee
MTRH	Moi Teaching and Referral Hospital
RAAS	Renin Angiotensin Aldosterone System
UACR`	Urinary Albumin-to-Creatinine Ratio
WHO	World Health Organization

OPERATIONAL DEFINITION OF KEY TERMS

Type 2 Diabetes mellitus

For the purpose of this study, a patient with diabetes was one who at the time of diagnosis was >30 years old and managed on oral hypoglycemic agent and/or insulin and/or diet; any patient above 18 years of age who was managed exclusively on oral hypoglycaemic agents without insulin was considered type 2 diabetes mellitus.

Newly diagnosed patient with type 2 diabetes mellitus

A patient diagnosed with type 2 diabetes mellitus for a period of up to two years, and not less than three months from the diagnosis.

Persistent Albuminuria

Spot Urine-Albumin-Creatinine-Ratio (UACR) of more than 30mg/24 hour, done on two separate occasions two to three months apart.

Hypertension

Based on the Eighth Joint National Committee (JNC 8) guidelines, any blood pressure measurement of ≥140/90 mmHg was considered high.

Known hypertensive patient

A known hypertensive patient was a patient who reported history of hypertension which was also confirmed from the patients file.

Glycaemic control

This was based on glycemic targets as recommended by American Diabetic Associations (ADA). HbA1c ≤ 7% and fasting blood sugar ≤ 7.2mmol/l was considered on target.

Lipid profile

The cut offs were based on ADA guidelines and the following figures were considered high: Low Density Lipoprotein (LDL) >2.6mmol/l; Triglycerides >1.7mmol/l; Total Cholesterol >5.17mmol/l and HDL was considered low if <1.29mmol/l for female, and <1.03mmol/l for male patients.

Body Mass Index (BMI)

This was calculated as weight in kg ÷ height in m^2. The degree of obesity was classified based on National Institute of Health (NIH) cut offs as follows;

Underweight (BMI of<18.5); Normal (BMI of 18.5-25); Overweight (BMI of 25-30); Obese (BMI of >30)

Waist circumference

The waist circumference was considered abnormal if >88cm for female and >102cm for male patients.

Estimated Glomerular Filtration Rate (eGFR)

This was calculated using chronic kidney disease -Epidemiology Collaboration (CKD-EPI) formula which was preferred based on a local study done in MTRH that showed that it was superior to other equations for estimating eGFR. Though the patients were only classified as eGFR above 60ml/min/1.73m^2; 30-60ml/min/1.73m^2 and <30ml/min/1.73m^2, mean eGFR of >90 ml/min/1.73m^2 was considered high.

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CHAPTER ONE

INTRODUCTION

Background:

Globally, there were 366 million people with diabetes Mellitus (DM) in 2011, and this is expected to rise to 552 million by 2030 (Whiting et al., 2011). Prevalence of DM is high in low and middle-income countries and is expected to increase over the next 19 years (Whiting et al., 2011). World Health Organization (WHO) 2010 report, indicates that type 2 DM is common between the ages of 34 and 65 years in developing countries although it affects older population in developed countries (Beaglehole, 2004). The International Diabetic Federation (IDF) estimated the prevalence of about 9.1% in Asia in the year 2010 (Shaw et al., 2010). In Sub Saharan Africa (SSA), type 2 DM accounts for over 90% of DM and its prevalence ranged from 1% in rural Uganda to 12% in urban Kenya (Hall et al., 2011). The Kenya Diabetes Management and Information (DMI) Center estimates the prevalence of diabetes mellitus in Kenya to be between 2.7% in rural areas and 10.7% in urban areas (Jalang, 2006).

According to the American Diabetes Association (ADA) 2014 guidelines, the criterion for diagnosis of diabetes mellitus includes: Glycosylated Haemoglobin (HbA1c) ≥6.5% or Fasting plasma glucose (FPG) ≥126mg/dL (7.0mmol/L); or 2-hour plasma glucose ≥200mg/dL (11.1mmol/L) during an Oral Glucose Tolerance Test (OGTT); or a random plasma glucose ≥200 mg/dL (11.1mmol/L) (American Diabetes Associations, 2014).

Although the pathophysiology of type 1 and 2 DM differ between the types, most of the long term complications such as micro vascular and macro vascular disease are similar in

nature. As the prevalence of diabetes mellitus is rising, so is the prevalence of the related long term complications as a result of micro and macro vascular diseases including diabetic nephropathy which is a serious micro vascular complication of DM that affects approximately one third of patients with DM (Gross et al., 2005).

DM is the leading cause of kidney disease in patients starting renal dialysis and affects approximately 20-40% of type 1 and type 2 DM patients (Gross et al., 2005). 1n 1983, Mogensen *et al.,* categorized diabetic nephropathy and emphasized on incipient diabetic nephropathy, which he first described as the forerunner of clinical diabetic nephropathy, and whose main presentation is abnormally elevated albumin excretion in urine, as measured by radioimmunoassay (Mogensen et al., 1983).

The global prevalence of albuminuria among type 1 and 2 DM patients currently stands at 39% (Parving et al., 2006). The prevalence of albuminuria among type 2 DM patients in Asia is about 39.8% with 40% of type 2 DM patients in Taiwan (Ibrahim et al., 2012). In SSA the prevalence of albuminuria is not well documented, and has been done in few countries with assistance of World Health Organization (WHO). These studies showed the prevalence of albuminuria among type 2 DM ranged from 10% in Tanzania in 2006, 26% in Kenya in 2002, 37% in Ethiopia in 1997 and 57% in Nigeria in 2006 (Tesfaye, & Gill, 2011).

Diabetic nephropathy is a process that over time progresses gradually over from albuminuria ranges >30-300 mg/24hr to albuminuria ranges >300mg/24hr which is overt nephropathy (Adler et al., 2003). Persistent albuminuria is an important marker for early diabetic nephropathy and its screening is emphasized by American Diabetic Associations

(ADA) recommendations (Expanded ABC,s) where B means blood pressure control/screening for albuminuria (Abbate, 2003). Type 2 DM patients can present for the very first time with complications including diabetic nephropathy because diabetes is really present for many years before the diagnosis is made (Agaba et al., 2004: Harris et al., 1992). Hence, patients with type 2 DM can present at diagnosis with albuminuria because of delays in diagnosis and other factors affecting protein excretion (American Diabetes Associations, 2014).

Screening for albuminuria among patients with type 2 DM should be done immediately after diagnosis of diabetes as up to 7% of them present with albuminuria at diagnosis (Adler et al., 2003). If albuminuria is recognized on time, early intervention can retard the progression to overt nephropathy. This can be done through control of risk factors of albuminuria which include; hyperglycemia, hypertension, dyslipidemia, and treatment with Angiotensin Converting Enzymes Inhibitors (ACEI's) or Angiotensin Receptor Blockers (ARB's).

This study therefore sought to determine the prevalence of persistent albuminuria and associated clinical characteristics of patients newly diagnosed with type 2 DM.

Problem Statement

Type 2 diabetes mellitus is a major health problem in Western Kenya. It is associated with micro vascular complications such as diabetic nephropathy and macro vascular complications. Diabetic nephropathy is the leading cause of end stage renal disease. It is manifested by persistent albuminuria which is a known early marker of diabetic nephropathy and increase cardiovascular morbidity and mortality. It is estimated up to a

third of the patients undergoing chronic dialysis at MTRH have diabetes mellitus. It is known that in resource limited-settings, the cost and access of dialysis and renal transplantation is restrictive and expensive. This has contributed to increased morbidity and mortality, and likely a reduction in economic productivity among the affected patients. However, among type 2 DM patients, there is no routine screening for persistent albuminuria in most health care facilities in Kenya including MTRH, hence, the prevalence of persistent albuminuria is not known.

Justification

Persistent albuminuria is an early marker of diabetic kidney disease and therefore is important in management of diabetic nephropathy. Diabetic nephropathy in its early stages is amenable to therapy using ACEI or ARBs. Early and routine screening of persistent albuminuria is cost effective to treat to prevent presentation of overt diabetic nephropathy and associated complications. This could lead to reduction in morbidity and mortality. Early screening will also reduce the overall cost of healthcare and improve the quality of life among patients with type 2 DM.

There are no published studies in our set up. Hence, the findings can be used in improving case management of patients with type 2 DM and design protocols to include screening for albuminuria at diagnosis. This study can serve as a baseline reference for development of other related studies.

Research questions

1. What is the prevalence of persistent albuminuria among the patients newly diagnosed with type 2 DM at MTRH?

2. What are the socio-demographics, clinical and laboratory characteristics of patients newly diagnosed with type 2 DM at MTRH?

3. What are the associations between persistent albuminuria and clinical, laboratory and socio-demographic characteristics among these patients?

Objectives

1.5.1 Broad Objective

To determine the prevalence and associated clinical characteristics of persistent albuminuria among patients newly diagnosed with type 2 DM at MTRH.

1.5.2 Specific Objectives

1) To determine the prevalence of persistent albuminuria among patients newly diagnosed with type 2 DM presenting at MTRH.

2) To describe the socio-demographic, clinical and laboratory characteristics of patients newly diagnosed with type 2 DM.

3) To determine the association between persistent albuminuria and clinical, laboratory and socio-demographic characteristics of the patients.

CHAPTER TWO

LITERATURE REVIEW

2.1 Prevalence of albuminuria among patients with diabetes mellitus

Various studies in different countries have found varied prevalence of albuminuria. Australian diabetes' obesity and lifestyle study carried out in 2004 found that the prevalence of albuminuria increases with the rise in glycaemia among 11,247 known and newly diagnosed type 1 and 2 DM adults. They found a prevalence of 5.1% among patients with normal glucose tolerance; 9.3% among those with impaired fasting; 11% among those with impaired glucose tolerance; 17.8% among patients newly diagnosed with type 1 and 2 DM; and 32.6% among known type 2 DM patients. This is consistent with the fact that albuminuria is common among known diabetic patients which could be present before the diagnosis of diabetes or/and the worsening of glucose tolerance (Dunstan et al., 2002).

The PROSIT (Proteinuria Screening and Intervention) project in Germany's primary care centers in 2000 carried out home self test for albuminuria among 569 type 1 and 2 DM patients. This result was later confirmed by a clinician and showed that the prevalence of albuminuria was 10.8% among type 2 DM (Gozzoli et al., 2000). Another study done in India between 2008 and 2009 showed 17.34% prevalence of albuminuria among 300 type 2 DM patients diagnosed within a period of 6 months. The study found that albuminuria was strongly associated with high blood pressure and male sex (Agarwal et al., 2011).

In Jos, Nigeria, the prevalence of albuminuria in 65 patients newly diagnosed with type 2 DM in a 2004 study was 49.2% and was strongly associated with systolic hypertension and retinopathy (Agaba et al., 2004). A study done in Kenya in 2002 at Kenyatta National Hospital (KNH) among 100 newly diagnosed type 2 DM patient found the prevalence of spot albuminuria was 26% (Wanjohi et al., 2002). Another KNH study done by Mwendwa *et al.,* in 2005 revealed that the prevalence of spot albuminuria was 25% for the ranges >30-300mg/24hr and 1% for ≥ 300mg/24hr (Mwendwa et al., 2005).

2.2 Risk factors of albuminuria

Studies done suggest that there is a link between albuminuria and age, sex, race, hyperglycemia, dyslipidemia, hypertension, obesity, and genetic factors (Stratton et al., 2006: Stratton et al., 2000). A cross sectional analysis to describe the prevalence and risk factors for albuminuria among African Americans with newly diagnosed type 2 DM was carried out in Atlanta Georgia clinic, among 1,167 patients with type 2 DM of duration of 2 years or less after diagnosis. It found a high prevalence of albuminuria (23%) among this population. Risk factors associated with albuminuria included; male sex, poor glycemic control, endogenous hyperinsulinemia, high blood pressure, elevated triglyceride levels and obesity (Kohler et al., 2000).

Several studies have shown that strict glycaemic control decrease the microvascular complications in patients with type 2 DM, hence delay onset of albuminuria. The Kumamoto study which was an eight year prospective study of Japanese patients showed that strict glycaemic control decreases the risk of microvascular disease in patients with type 2 DM (Sun et al., 2011). Also the Diabetes Control and Complications Trial (DCCT) suggested that intensive therapy can prevent or delay the microvascular complications of

DM (Worrall, 1994). In addition, the Action in Diabetes and Vascular Disease: Preterax and Diamicron MR Controlled Evaluation (ADVANCE) which was also a prospective study on patients who were either on intensive glucose control group (HbA1c≤6.5%) verses standard control group (HbA1c≤7.3%) showed a reduction of microvascular and macrovascular outcomes in the intensive control arm (The ADVANCE Collaborative group, 2008). Conversely, the Action to Control Cardiovascular Risk in Diabetes (ACCORD), a parallel-group randomized clinical trial done in 77 clinical sites in North America, showed that intensive glycemic control was associated with mortality as a result of hypoglycemia and the study was terminated early (Hoogwerf, 2008). However, analysis that was done on the same study subjects revealed that intensive glucose control delayed the onset of albuminuria (Ismail-Beigi et al., 2010).

Haneda *et al*. Japanese study found that hypertension was the risk factor for microalbuminuria among non insulin dependent diabetic patients (Haneda et al., 1992). A United Kingdom Prospective Diabetic study (UKPDS) that was done in 23 hospital based clinics in England, Scotland and Northern Ireland, showed association between systolic hypertension and microvascular complications (Adler et al., 2000). Another study done in Edinburg, Scotland in 1990 proved that there is association between age and albuminuria, this study assessed the prevalence of albuminuria in 149 newly diagnosed type 2 DM participants who were followed up for one year, 16% of the patients with persistently high UACR were elderly population (Patrick et al., 1990). A large nationally representative cohort study, Third National health and Nutrition Examination Survey (NHANESII:1988-1994), which involved 5,659 participants found out that albuminuria and metabolic syndrome (high waist circumference, high triglycerides, low HDL, high

total cholesterol, high blood pressure, and high fasting glucose) had an association (Palaniappan et al., 2003). Also another study showed that dyslipidemia is associated with microvascular complications including diabetic nephropathy (Agrawal et al., 2006).

Genetic factors have been associated with diabetic nephropathy.1n 1998, a study was done to investigate the association between genetic polymorphisms in apolipoprotein E and the progression of diabetic nephropathy in patients with type 2 DM over a 10-year period, indicated that apolipoprotein E polymorphism was associated with the progression of diabetic nephropathy. Presence of the apolipoprotein E4 allele was a protective factor, and other alleles were risk factors (Kimura et al., 1998). Meta-analysis of 18 studies done in 2012 showed that peroxisome proliferator-activated receptor γ polymorphism Pro12Ala is associated with nephropathy in type 2 DM. In the overall analysis, the Ala12 variant was observed to be significantly associated with decreased risk in diabetic nephropathy (odds ratio 0.76 [95% CI 0.61-0.93]) (Zhang et al., 2012).

2.3 Pathogenesis of diabetic nephropathy

Dronavalli *et al.* concluded that multiple mechanisms contribute to the development of diabetic nephropathy such as an interaction between hyperglycemia-induced metabolic and hemodynamic changes and genetic predisposition which sets the stage for kidney disease (Dronavalli et al., 2008). Hemodynamic changes involve the activation of renal tissue Renin Angiotensin and Aldosterone System (RAAS) and increased expression of growth factors have been demonstrated at early stages of the disease (Rincon-Choles et al., 2002) High levels of glucose can account for synthesis of extracellular matrix proteins that lead to thickening of the basement membrane and glomerulosclerosis (Wardle, 1996). Another glucose product, glucosamine-6-phosphate, is formed when

there is hexosamine flux along with insulin resistance in tissues, and is implicated in glomerulosclerosis (Wardle, 1996). Metabolic pathway, leads to nonenzymatic glycosylation, increased protein kinase C (PKC) activity, and abnormal polyol metabolism (Hamada et al., 1996).

Mongensen *et al.* classified diabetic nephropathy into 5 stages: Stage 1 is characterized by early hyperfunction and hypertrophy. Stage 2 develops silently over many years and is characterized by morphologic lesions without signs of clinical disease. Stage 3, incipient diabetic nephropathy, is the forerunner of overt diabetic nephropathy. Its main manifestation is albuminuria at ranges 30-300mg/24 hr. A slow, gradual increase over the years is a prominent feature in this very decisive phase of renal disease in diabetes when blood pressure is rising. The increased rate in albumin excretion is higher in patients with increased blood pressure. Stage 4 is overt diabetic nephropathy, the classic entity characterized by persistent proteinuria (greater than 0.5 g/24 hr). Stage 5 is ESRD with uremia due to diabetic nephropathy (Mogensen et al., 1983).

Tervaert *et al.* classified diabetic nephropathy based on biopsies as follows: Class I, glomerular basement membrane thickening: isolated glomerular basement membrane thickening and only mild, nonspecific changes by light microscopy that do not meet the criteria of classes II through IV. Class II, mesangial expansion, mild (IIa) or severe (IIb): glomeruli classified as mild or severe mesangial expansion but without nodular sclerosis (Kimmelstiel-Wilson lesions) or global glomerulosclerosis in more than 50% of glomeruli. Class III**,** nodular sclerosis (Kimmelstiel-Wilson lesions): at least one glomerulus with nodular increase in mesangial matrix (Kimmelstiel-Wilson) without changes described in class IV. Class IV, advanced diabetic glomerulosclerosis: more

than 50% global glomerulosclerosis with other clinical or pathologic evidence that sclerosis is attributable to diabetic nephropathy. A good inter observer reproducibility for the four classes of diabetic nephropathy was shown (interclass correlation coefficient = 0.84) in a test of this classification (Tervaert et al., 2010).

2.4 Diagnosis of diabetic nephropathy

Diabetic nephropathy can be diagnosed by screening for albuminuria and this can be performed by three methods: 1) Measurement of the Urine-Albumin-to-Creatinine Ratio (UACR) in a random spot collection which was used in this study ; 2) 24-hour urine collection with creatinine, allowing the simultaneous measurement of creatinine clearance; 3) or/and timed (e.g., 4-hour or overnight) collection (Abbate, 2003).

Measurement of the UACR gives a fairly accurate estimate of the 24 hour albumin excretion in mg/day in a random spot collection. It is the easiest to carry out in an office setting, cost effective and provides accurate information (Guy et al., 2009: Kohler & Krämer, 1994: Parikh et al., 2002). However, exercise within 24 hours, infection, fever, heart failure, marked hyperglycemia and marked hypertension may elevate urinary albumin excretion over baseline values (Gross et al., 2005).

Traditionally albumin excretion was classified as micro albuminuria (30-300mg/24 hr) and macro albuminuria (>300mg/24 hr). However, the 2014 ADA guidelines recommends the use of persistent albuminuria at levels 30-300mg/24 hr and >300mg/24 hours. This new nomenclature is used to emphasize the nature of continuous albuminuria as a risk factor (American Diabetes Associations, 2014). Normal albumin excretion is

defined as albumin excretion <30mg/24 hr. According to ADA, it is recommended that test of albumunuria be repeated within 3-6 months to confirm for persistence.

2.5 Prevention and treatment of diabetic nephropathy

According to the ADA standard of care guidelines to reduce the risk or slow the progression of nephropathy includes the following;

The first recommendation is optimization of glucose control, by patient's self monitored blood glucose (SMBG) which refers to a series of tests done by the patient at home at different times using home based glucometers, also by using the blood HbA1c which measures average glycaemic control over the previous 2 – 3 months which can be used to assess treatment efficacy (American Diabetes Associations, 2014: Inzucchi et al., 2012).

The second recommendation is optimization of blood pressure control with previous guidelines recommending a target of less than 130/80mmHg. However, the 2014 ADA guidelines recommend a target BP of less than 140/90 mmHg (American Diabetes Associations, 2014). In addition, the 2014 Evidence-Based Guideline for the Management of high Blood Pressure in Adults, Eighth Joint National Committee (JNC 8) recommend a BP goal of ≤ 140/90mmHg among diabetic hypertensive patients (James et al., 2014).

The third recommendation is to perform an annual test to assess urine albumin excretion in all type 2 DM patients, starting at diagnosis. This is based on various studies that proved that screening for diabetic nephropathy must be started at the time of diagnosis in patient with type 2 DM since about 7% of them already have albuminuria at that time (Adler et al., 2003). Early detection of albuminuria and the introduction of a

multifactorial treatment strategy make it possible to delay progression of diabetic nephropathy (Gross et al., 2005: Henrichs, 1991: Craig et al., 2003).

The fourth recommendation is measurement of serum creatinine, at least annually in all adults with diabetes regardless of the degree of urine albumin excretion. The serum creatinine should be used to estimate GFR and stage the level of chronic kidney disease (CKD), if present.

ADA recommendations for secondary prevention of diabetic nephropathy apart from the above, is the use of either Angiotensin Converting Enzyme Inhibitors (ACE-I) or Angiotensin Receptor Blockers (ARBs) and reduction of protein intake may improve measures of renal function. Several studies have shown that RAAS blockade slows or retards the progression of albuminuria in type 2 DM and it mainly benefits patients with hypertension and/or albuminuria (Strippoli, et al., 2005: Chawla et al., 2010: Ruggenenti et al., 2010: Fried et al., 2013). However not all patients with type 2 DM are hypertensive and may not routinely benefit from an ACE-I or ARB in our setup due to poor screening. Furthermore, the use of ACEI/ARB for primary prevention of diabetic nephropathy in normotensive patients is not recommended (American Diabetes Associations, 2014).

From available literature, persistent albuminuria is an important risk factor for nephropathy and cardiovascular mortality. Early detection and initiation of measures to reduce albuminuria would improve on diabetic long range complications.

Current literature above shows that measuring of persistent albuminuria in newly diagnosed diabetics in resource- limited setting is not routinely being done.

CHAPTER THREE

RESEARCH METHODOLOGY

3.1 Study site and setting

This study was done in the medical and diabetic outpatients' clinics at Moi Teaching and Referral Hospital (MTRH). The Hospital is located in Eldoret town, which is 350 Kilometers northwest of the Kenyan capital, Nairobi. MTRH is a tertiary (level 6) health facility serving as a teaching hospital for Moi University School of Medicine, Public Health, Nursing and Dentistry. Others include Kenya Medical Training Center (KMTC), Eldoret and University of Eastern Africa Baraton School of Nursing. MTRH is also a training center for medical, clinical and nursing officer interns. It is the referral hospital which has a catchment population of approximately 13 million people. The population is cosmopolitan, as it serves counties in western and north rift region comprising of both urban and rural areas.

3.2 Study population

All type 2 diabetes mellitus patients. Type 2 DM was confirmed by reviewing the patients' medical history and their blood sugars as per the definition. The target population was patients who presented to medical and diabetic clinics. The sampled population was the ones who consented to participate in the study and met the inclusion criteria.

3.3 Study Design

This study was a cross sectional descriptive study. All patients who had albuminuria were seen on two occasions at least 2-3 months apart to document persistence of albuminuria.

3.4 Sampling and recruitment

3.4.1 Sampling technique

Patients meeting the study inclusion criteria were recruited through consecutive sampling after initial screening of all type 2 diabetic mellitus patients who presented to the medical and diabetic clinics.

3.4.2 Eligibility criteria

3.4.2.1 Inclusion criteria

1. Newly diagnosed patients with type 2 diabetes mellitus as per the case definitions, aged 18 years and above.

3.4.2.2 Exclusion criteria

1. Patients with fever
2. Patients who presented with Urinary Tract Infection (UTI)
3. Patients with heart failure
4. Known patients with chronic kidney disease
5. Critically ill patients

3.4.3 Sample size

This was derived using the Fisher *et al*, (1998) formulae

$$n = \frac{Z^2_{(1-\alpha/2)} \cdot p(1-p)}{d^2}$$

Where;

n = sample size;

Z = the value corresponding to 95% confidence =1.96

α = Significance level at 5% = 0.05

p = Estimated prevalence of albuminuria among type 2 diabetes mellitus patients

d = margin of error

In a study that was done in KNH in 2002 in almost similar setting to MTRH, the prevalence of albuminuria in patients newly diagnosed with type 2 DM was 26% at the confidence interval of 95%.

Therefore,

$$n = \frac{1.96^2 \times 0.26 \times 0.74}{0.05^2}$$

N= 296

According to the latest data for the year 2010 at MTRH, approximately 127 patients are seen annually, hence need for Finite population correction to account for small population. The desired sample size was 90 patients as shown by finite population correction formula below.

n=n/ (1+(n/N))

N= 296/ (1+296/127)

N= 90

3.5 Study procedures

As patients arrived in the two clinics, a nurse went through their medical charts to identify those who were eligible. Those who met the inclusion criteria had their files put aside for consenting. The principal investigator reviewed the sampled charts and approached the identified patients. Those who met the inclusion criteria had their consent obtained. They were informed that they were being screened for albuminuria and if they were found to be having albuminuria they would require a repeat test within 2-3 months for confirmation. They were also informed about the other laboratory tests that were to be done. If they weren't fasted, then they were rescheduled for another day.

The patients' self reported bio data and a comprehensive medical history of current and past illnesses, including but not limited to: duration of diabetes, history of hypertension, stroke, ischemic heart disease and cigarette smoking as well as medication use including diabetic medication, use of ACE inhibitors or ARBs, and lipid lowering agents were taken. Those who reported duration of diabetic illness less than 2 years but more than 3 months after diagnosis were considered newly diagnosed and were eligible.

Physical examination of all the study participants was performed. Patient's weight was taken and recorded to the nearest half kilograms (kg) using a standard weighing scale with the patients dressed in light clothing and without shoes. Height was taken against a vertical scale with the patient standing upright and without shoes and recorded to the nearest centimeter (cm). Body Mass Index (BMI) was then calculated as weight in kg /height in (m^2) and the degree of obesity was classified based on National institute of Health (NIH) cut offs (National Institutes of Health (NIH) National Heart, Lung, and Blood Institute, 1998).

Waist circumference was measured using the standard protocol by palpating the upper right hipbone of the patient until the uppermost lateral border of the iliac crest was located; where this was not palpable the greatest gluteal circumference was measured by applying tension to the tape to ensure it is snug, without causing indentation to the skin. This was taken at the end of a normal expiration and the measurement was approximated to the nearest 0.5cm.

Blood pressure measurement was done after a patient had rested for at least 5 minutes. Two readings were taken and the average was recorded in the questionnaire. Patients who reported history of hypertension, was verified from the records. These patients were classified either as good or poor control depending on the average blood pressure readings of their last visit and the day of enrollment. The patients who were not known hypertensive but with BP readings of systolic above 140mmHg and diastolic of more than 90mmHg had their charts reviewed and if they had high Blood pressure (>140/90mmHg during their last visit they were classified as newly diagnosed hypertensive.

Blood samples were obtained from patients following an overnight fast by finger pricking for FBS and HbA1c (Appendix IV and V). HbA1c $\leq$ 7% and fasting blood sugar $\leq$ 7.2mmol/l was considered to be on target. Blood samples were drawn and 2mls put in an plain bottle for total cholesterol, LDL, HDL and triglycerides (Appendix VII). A second 2mls blood sample was taken for determination of serum creatinine (Appendix VIII). The procedure for drawing blood is explained in Appendix VI.

Random spot urine was taken for estimation of the 24 hr urine albumin based on urine-albumin-creatinine ratio. A routine urinalysis by dipstick was also done. Those who had features of UTI based on the presence of nitrites and leucocytes were excluded from the study but informed of the results and their sample send for microscopy and culture and referred for treatment.

The patients who were found to have albuminuria of ≥30mg/24hr had a repeat of both urine albumin creatinine ratio and dipstick urinalysis two to three months apart. Persistent Albuminuria was considered to be present if positive results were obtained on two separate occasions (refer to appendix IX).

The estimated glomerular filtrate (eGFR) was calculated using the CKD-EPI (Chronic Kidney Disease-Epidemiology Consortium) equation, 32788 * min (scr/k,1)^a * max (scr/k,1)^-1.209 * 0.993^age * 1.018 if female. Where Scr is serum creatinine (mg/dL), k is 0.7 for females and 0.9 for males, a is –0.329 for females and –0.411 for males, min indicates the minimum of Scr/k or 1, and max indicates the maximum of Scr/k or 1. This formula was preferred based on the recent local study which showed that CKD-EPI was superior to other equations for the estimation of eGFR among HIV infected patient in MTRH (Wyatt et al., 2013). This was however true for patients with eGFR of more than 90ml/min/1.73 m^2. Though there is no evidence to suggest this applies to all patients, this is the only formula that has been validated locally.

All laboratory investigations were carried out at the MTRH laboratory according to good laboratory and clinical guidelines/practices. The procedures were carried out by the principal investigator, research assistant and a trained laboratory technician.

3.6 Data collection and management

3.6.1 Data collection

Data was collected between January and December 2013, using interviewer administered structured questionnaire (Appendix I). The data collection tool was validated by monitoring the data trends during data collection. Medical records were also reviewed and relevant clinical and laboratory data were obtained and entered into the data collection form. The variables collected included demographic characteristics such as age, gender, and occupation. Medical and family history of hypertension, diabetes and kidney disease were also obtained. Other variables collected included; laboratory parameters (HbA1c, FBS, creatinine, lipid profile, Urinalysis and UACR). The dependent/outcome variables are the levels of albuminuria for the patients newly diagnosed with type 2 DM. The data was later double-entered into a computer Microsoft Access database and pass-worded.

3.6.2 Data analysis and presentation

Data analysis was done using STATA version 13 special edition. Categorical variables were summarized as frequencies. Continuous variables that followed normal distribution were summarized as mean. Otherwise they were summarized as median and the corresponding inter quartile range (IQR) (if they were skewed). The test for association between categorical variables was done using Pearson's Chi Square test while that for associations between categorical and continuous variables was done using the two sample Wilcoxon rank sum test if the continuous variables violated the assumptions of normal distribution. The test for difference in mean between two continuous variables was done using the two sample t test when the variables did not violate the assumptions of normal

distribution. Comparison of a median value to the null value was done using a sign test while a comparison of a mean value to the null value was done using one sample t-test. We reported the corresponding p-values ($p < 0.05$ was considered significant). Data was presented in form of tables and graphs.

3.7 Ethical considerations

This study was carried out with the approval of the Institutional Research and Ethics Committee (IREC) of MTRH and Moi University School of Medicine and permission from MRTH management. A signed informed consent was obtained for each participant who was included in this study (Appendix II). Confidentiality was maintained throughout the study by pass-wording database and limiting its access only to principal investigator and research assistants. Interviews were carried out in a consultation room to ensure privacy and convenience. All participants including those who declined consent received the same level of care awarded to all other patients irrespective of their participation. There were very minimal anticipated risks to the participants attributable to this study except the physical pain and discomfort associated with sample collection. Questionnaires will be shredded after three years or publication of the study findings. There was no conflict of interest in this study and no incentives were used to recruit patients. Patients were informed of their results and the same availed to their primary clinicians. This thesis shall be availed at the MUSOM library. It will also be published in a reputable journal and presented in professional conferences and seminars.

CHAPTER FOUR

RESULTS

4.1 Screening and enrollment into the study

A total of 205 type 2 DM patients were screened at the diabetic and medical outpatient clinics in MTRH between January and December 2013 of which 92 patients were enrolled. The details are shown in figure 1.

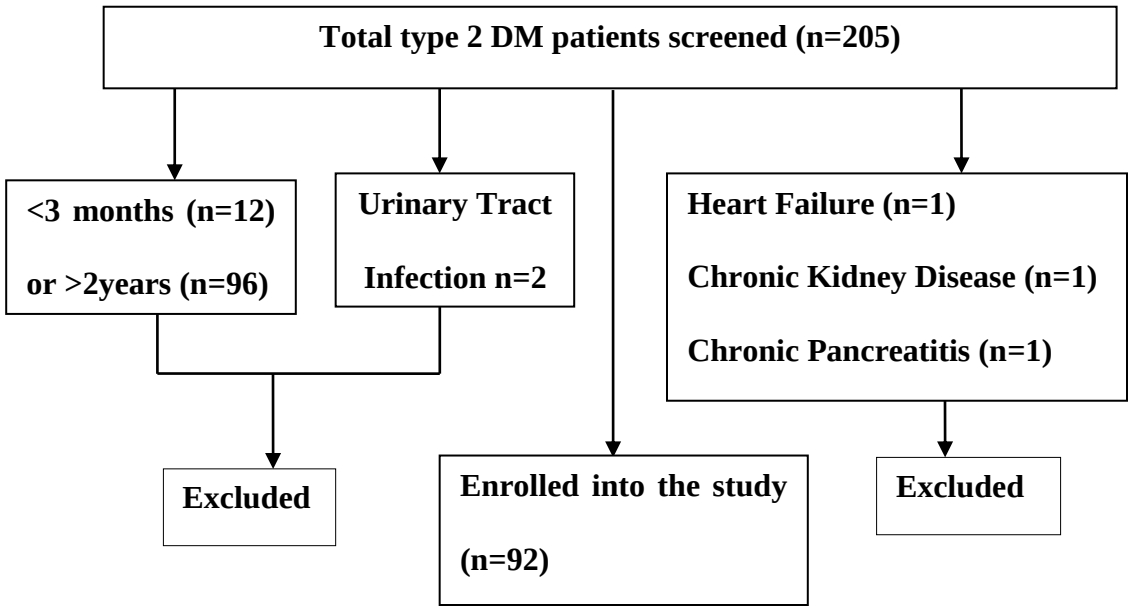

Figure 1: Enrollment Schema

4.2 Prevalence of albuminuria

The prevalence of persistent albuminuria was 18.5% (17/92) and the prevalence of spot albuminuria was 29.4 % (27/92). UACR was used to quantify albuminuria range in all patients and only those who had UACR of >30mg/24hr had a repeat test done between 2-3 months apart (Table 1).

Out of 92 patients, 14 (15%) had proteins on dipstick urinalysis. Among the 27 who had albuminuria during the first test, 9 (30%) had protein on dipstick.

Table 1: Albuminuria range of the patients

	Number	**Urine Albumin Levels (mg/24hr)**	**N (%)**
UACR1	92	<30	65 (71%)
		30-300	25 (27%)
		>300	2 (2%)
UACR2	27	<30	10 (37%)
		30-300	15 (56%)
		>300	2 (7%)

4.3 Socio-demographic characteristics of the patients

The patients' age ranged from 30 to 89 years with a mean age of 55 years (SD 12.5 years) and the most frequent age group being 50-59 years (Figure 2).

Females were the majority, comprising 53 (58%) of the patients. Most patients 41 (49%) were unemployed, 34 (37%) were self employed and 17 (18%) were employed. A few, 15 (16.3%) reported history of smoking cigarettes.

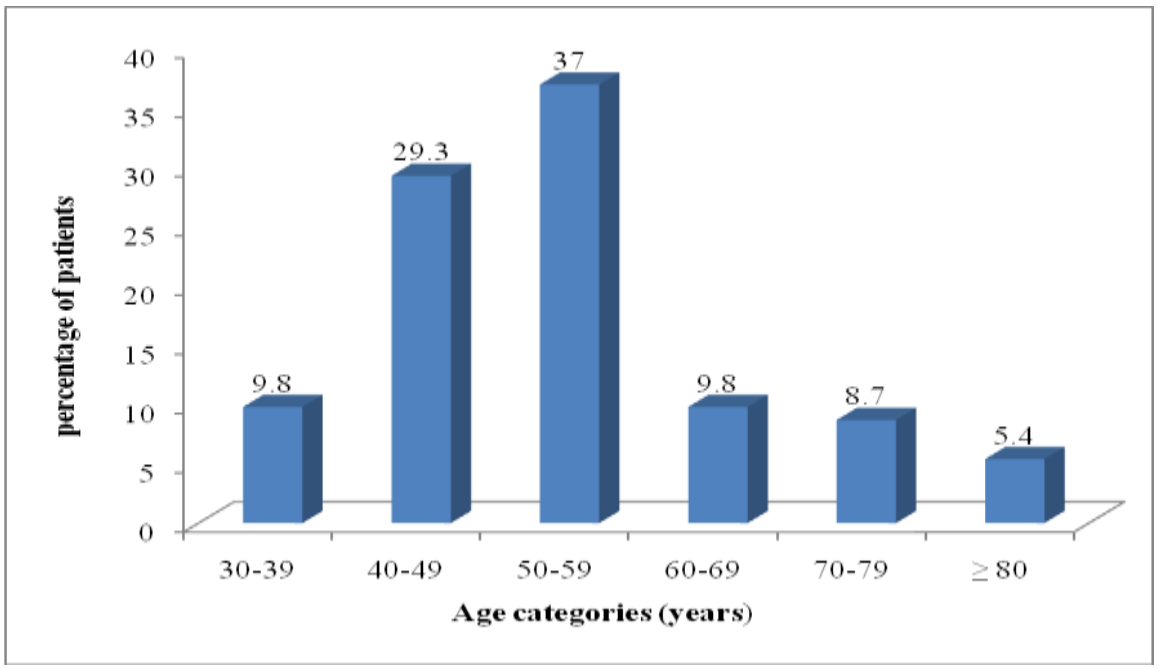

Figure 2: Age categories of the patients

4.4 Clinical characteristics of the patients

4.4.1 Medical history of the patients

The median duration of illness was 9 months (IQR 3-14). Out of the 92 patients, 44 (47.8%) reported history of hypertension, 4 (4.4%) stroke and 2 (2.2%) diabetic retinopathy. None of the patients reported history of ischemic heart disease. Family history of diabetes was reported by 43 (47%) patients and of kidney disease by 1 (1.1%).

Diabetes was managed mainly by oral hypoglycemic agents among 66 (71.7%), and insulin 22 (23.9%) while 3 (3.3%) were managed with both oral hypoglycemic agents and insulin. One patient was managed by diet.

The other category comprised of seven patients using the following combination: 1) glibenclamide, insulin and metformin; 2) glimepiride, metformin and insulin; 3)

glimepiride and pioglitazone; 4) Insulin and metformin; 5) Insulin and Pioglitazone; 6) and two patients who were on glibenclamide alone. (Figure 3)

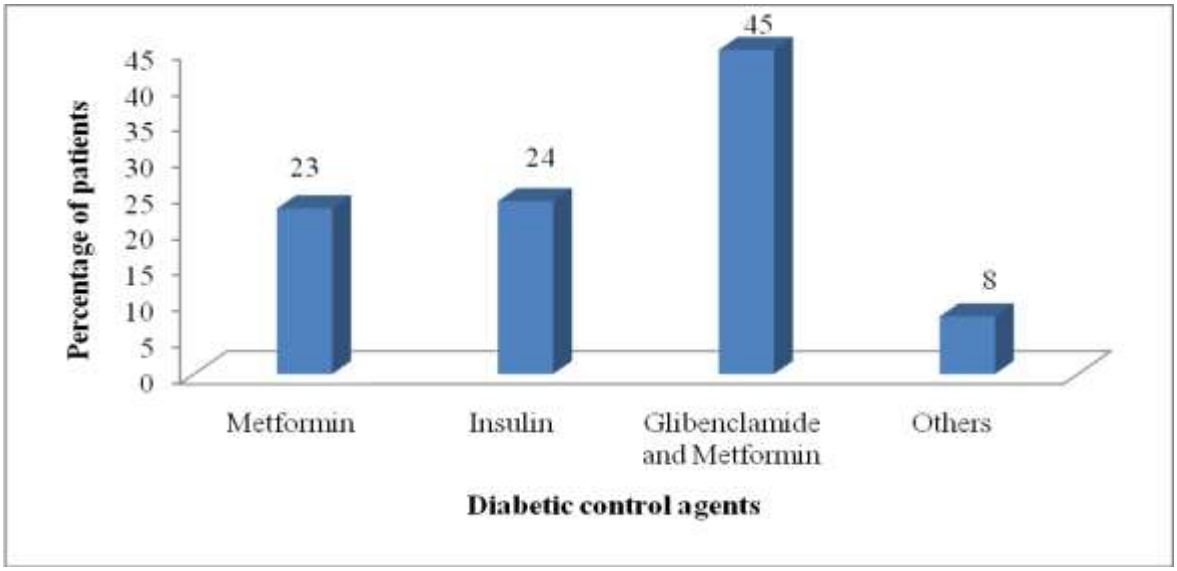

Figure 3: Diabetic control agents used by the patients

Among 44 patients with history of hypertension, 35 (80%) were on at least one antihypertensive drug while 9 (20%), were not on any antihypertensive drugs. The antihypertensive agent used was mainly ACEI's or ARB's (56.8%). Five (10%) patients without history of hypertension were on enalapril.

Table 2 shows the number of patients who were on ACEI's and ARB's. Out of 30 patients, 24 (80%) were on enalapril, 5 (16.7%) on lorsartan while 1 (3.3%) was on telmisartan.

Eight (8.7%) patients were on lipid lowering agents, mainly atorvastatin (87.5%) and resuvastatin (12.5%).

Table 2: Medical history of the patients

Variable	Numbers (n=92)	Percentages
Reported history of hypertension	44	47.8%
Reported history of stroke	4	4.4%
Reported family history of diabetes	43	47%
Reported family history of kidney disease	1	1.1%
Reported history of diabetic retinopathy	2	2.2%
Reported glycemic control agents		
Oral hypoglycemic agents	66	71.7%
Insulin	22	23.9%
Both insulin & oral hypoglycemic	3	3.3%
Diet control	1	1.1%
Reported use of ACEIs/ARBs	30	32.6%
Reported use of Statins	8	8.7%
Reported use of Aspirin	6	6.5%

4.4.2 Clinical characteristics of the patients

Majority of the patients 89 (96.7%) had normal physical examination findings; 2 had mild pedal edema while one had left sided hemiparesis as a result of stroke. The mean body mass index (BMI) was 26.9 (SD: 4.9) Kg/m^2 and the mean waist circumference was 100.8cm. The mean systolic blood pressure (SBP) was 133 (SD: 24) and mean diastolic blood pressure (DBP) was 79 (SD: 11). The mean pulse rate was 87 (SD: 15) beats per minute.

The mean systolic blood pressure (SBP) and mean diastolic blood pressure (DBP) was 146 (SD 27) mmHg and 84 (SD 12) mmHg respectively among patients with history of hypertension. Similarly, among those without history of hypertension, the mean SBP and DBP were 122 (SD 13) mmHg and 76 (SD 9) mmHg, respectively.

Among this group of known hypertensive, 24 (55%) and 2 (4%) patients with no history of hypertension were hypertensive (Bp>140/90mmHg) at the time of the study.

4.4.3 Laboratory characteristics of the patients

The median fasting blood sugar (FBS) was 8.5mmol/l(IQR: 5.9-12.1) with a mean HbA1c of 10.1%. The mean eGFR was 104.9 (SD 33.4) ml/min/1.73m^2 with majority of the patients having eGFR above 60ml/min/1.73m^2.

The mean total cholesterol was 5.1mmol/l slightly higher than the recommended value of 5.17mmol/l. Similarly the median LDL was 3.0mmol/l although the recommended LDL is 2.6mmol/l. The median triglycerides value was 1.7mmol/l (IQR: 1.4-2.5) and mean HDL for the male was 0.9mmol/l (SD: 0.3) while for the female was 1.0mmol/l (SD: 0.3). Other clinical and laboratory characteristics are summarized in Table 3

Table 3: Clinical and Laboratory characteristics of the patients

Variable	Mean (SD) or Median (IQR)	Number n=92	Percentage
Median duration of illness (months)	9 (3-14)		
BMI	26.9 (4.9)		
Underweight (<18.5)		3	3%
Normal (18.5-25)		31	34%
Overweight (25-30)		35	38%
Obese (>30)		23	25%
Waist circumference*	100.8 (15.2)		
Normal		35	38%
Abnormal		57	62%
Hypertension status			
≤140/90mmHg		66	71.7%
>140/90mmHg		26	28.2%
Systolic Blood Pressure	133mmHg (24mmHg)		
>140mmHg		25	27.2%
≤140mmHg		67	72.8%
Diastolic Blood Pressure	79mmHg (11mmHg)		
>90mmHg		14	15.2%
≤90mmHg		78	84.8%
FBS	8.5 (5.9-12.1)		
≤7.2mmol/l		38	41.3%
>7.2mmol/l		54	58.7%
HBA1c	10.1 (2.8)		
≤7%		16	17.4%
>7%		76	82.6%
LDL	3.0 (2.3-3.7)		
≤2.6mmol/l		35	38%
>2.6mmol/l		57	62%
Triglycerides	1.7 (1.4-2.5)		
≤1.7mmol/l		65	70.7%
>1.7mmol/l		27	29.4%
Total cholesterol	5.1 (1.2)		
≤5.17mmol/l		57	62%
>5.17mmol/l		35	38%
HDL**	1.0 (0.3)		
High		16	17.4%
Low		76	82.6%
Dyslipidemia present			
Yes		88	96%
No		4	4%
eGFR (ml/min/1.73m^2)	104.9 (33.4)		
Above 60		86	94%
30-60		4	4%
<30		2	2%

*Waist circumference; Normal (Female ≤88cm, Male≤102cm) Abnormal (Female >88cm, Male>102cm)
** HDL; High (Female≥1.29mmol/l, Male≥1.03mmol/l) Low <1.29mmol/l (Female), 1.03mmol/l (Male)

4.5. Associations between persistent albuminuria and patients' characteristics

4.5.1 Associations between persistent albuminuria and socio-demographics characteristics of the patients

There was no significant association between persistent albuminuria and demographic characteristics (p >0.05). This is summarized in Table 4.

Table 4: Association between persistent albuminuria and socio-demographic characteristics of the patients

Variable	Normal albuminuria n=75	Persistent albuminuria n=17	p-value
Mean age (years)	54 (11.6)	59 (15.4)	0.126[t]
Gender Male Female	 30 (40%) 45 (60%)	 9 (53%) 8 (47%)	 0.330
History of Smoking Yes No	 10 (13%) 65 (87%)	 5 (29%) 12 (71%)	 0.143[f]

Key " *f* " – *Fisher's exact test.* *"t" – Two sample t-test*

4.5.2 Association between persistent albuminuria and clinical characteristics of the patients

There were no statistically significant differences between persistent albuminuria and clinical findings. However, the results show the participants with persistent albuminuria were more likely to have hypertension, stroke, diabetic retinopathy, and were on lipid lowering agents

Patients with normal as well as abnormal albumin excretion levels tended to be overweight, pre-hypertensive, high pulse rate and their durations of illnesses was also similar. A higher proportion of participants with hypertension were found in the group with persistent albuminuria (Table 5 & 6)

Table 5: Association between persistent albuminuria and medical history of the patients

Variable	**Normal albuminuria (n=75)**	**Persistent albuminuria (n=17)**	**P-Value**
Reported history of hypertension			
Yes	35 (47%)	9 (53%)	0.640
No	40 (53%)	8 (47%)	
Reported history of stroke			
Yes	3 (4%)	1 (6%)	0.565[f]
No	72 (96%)	16 (94%)	
Reported family history of diabetes			
Yes	35 (47%)	8 (47%)	
No	40 (53%)	9 (53%)	0.977
Reported family history of kidney disease			
Yes	1 (1%)	0 (0%)	
No	74 (99%)	17 (100%)	1.000[f]
Reported history of diabetic retinopathy			
Yes	1 (1%)	1 (6%)	0.337[f]
No	74 (99%)	16 (94%)	
Diabetic control agents			
Insulin & oral hypoglycemic agents	2 (3%)	1 (6%)	
Diet control	1 (1%)	0	0.717
Insulin	18 (24%)	4 (24%)	
Oral hypoglycemic	54 (72%)	12 (71%)	
Use of ACEI/ARB			
Yes	26 (35%)	4 (24%)	0.376
No	49 (65%)	13 (76%)	
Use of lipid lowering agents			
Yes	6 (8%)	2 (12%)	0.637[f]
No	69 (92%)	15 (88%)	

Key; *"[f]" – Fisher's exact test "t"– Two sample t-test*

Table 6: Associations between persistent albuminuria and clinical characteristics of the patients

Variable	Normal albuminuria (n=75)	Persistent albuminuria (n=17)	P-Value
Mean BMI (Kg/m2)	26.8 (4.7)	27.1 (6.0)	0.833[t]
Mean Waist circumference (cm)	100.8 (15.5)	100.8 (13.7)	0.992[t]
Mean SBP (MM Hg)	132 (23)	138 (31)	0.420[t]
Mean DBP(MM Hg)	79 (11)	82 (10)	0.375[t]
Mean Pulse rate (beats per Min)	86 (15)	92 (16)	0.116[t]
Median duration of illness (months)	9 (3-15)	7 (4-12)	0.640
BMI			
Underweight (<18.5)	2 (3%)	1 (6%)	0.677[f]
Normal (18.5-25)	25 (33%)	6 (35%)	
Overweight (25-30)	30 (40%)	5 (29%)	
Obese (>30)	18 (24%)	5 (29%)	
Waist circumference*			
Abnormal	48 (64%)	9 (53%)	
Normal	27 (36%)	8 (47%)	0.396
SBP			
>140mmHg	20 (27%)	5 (29%)	
≤140mmHg	55 (73%	12 (71%)	0.772[f]
DBP			
>90mmHg	9 (12%)	5 (29%)	
≤90mmHg	66 (88%)	12 (71%)	0.126
Hypertension			
BP>140/90mmhg	20 (27%)	6 (35%)	0.553[f]
BP≤140/90mmHg	55 (73%)	11 (65%)	

Key; *" f " – Fisher's exact test "t"– Two sample t-test*

*Normal (Female ≤88cm, Male≤102cm) Abnormal (Female >88cm, Male>102cm)

4.6 Association between persistent albuminuria and laboratory findings

More participants with high HBA1c, LDL, triglycerides and Cholesterol had persistent albuminuria. Persistent albuminuria was positively associated with higher mean serum

creatinine and lower eGFR. Patients with higher eGFR were 2% less likely to have persistent albuminuria (OR=98: 95% CI: 0.96, 1.00, p=0.016) (Table 7).

Table 7: Association between persistent albuminuria and the laboratory findings of the patients

Variable	**Normal albuminuria(n=75)**	**Persistent albuminuria (n=17)**	**P**
Mean HBA1c	10.2 (2.8)	9.4 (2.8)	0.321[t]
Mean HDL	1.0 (0.3)	0.9 (0.2)	0.423[t]
Median LDL	3.0 (2.3-3.7)	3.1 (2.6-4.0)	0.560
Mean Total Cholesterol	5.0 (1.1)	5.3 (1.6)	0.338[t]
Median Triglycerides	1.7 (1.4-2.3)	1.9 (1.4-3.3)	0.337
Median Serum creatinine	63 (52-75)	73 (58-94)	0.029
Median FBS	8.1 (5.8-11.9)	8.6 (6.1-12.2)	0.960
Mean eGFR	118.6 (38.1)	93.3 (40.0)	0.016
Fasting Blood Sugar ≤7mmol/l >7mmol/l	 44 (59%) 31 (41%)	 10 (59%) 7 (41%)	0.991
HbA1c ≤7% >7%	 12 (16%) 63 (84%)	 4 (24%) 13 (76%)	0.485
LDL >2.50mmol/l ≤2.50 mmol/l	 44 (59%) 31 (41%)	 13 (76%) 4 (24%)	0.172
Triglycerides ≥1.8mmol/l <1.8mmol/l	 20 (27%) 55 (73%)	 7 (41%) 10 (59%)	0.236
Total cholesterol ≤5.17mmol/l >5.17mmol/l	 28 (37%) 47 (63%)	 7 (41%) 7 (59%)	0.768
HDL* High Low	 15 (20%) 60 (80%)	 1 (6%) 16 (94%)	0.288[f]
eGFR Above 60 30-60 15-30	 72 (96%) 3 (4%) 0	 14 (82%) 1 (6%) 2 (12%)	0.036[f]

Key ; *"f" – Fisher's exact test. "t" – Two sample t-test.*
* HDL; High (Female≥1.29, Male≥1.03) Low <1.29 (Female), 1.03 (Male)

CHAPTER FIVE

DISCUSSION

5.1 Prevalence of persistent albuminuria

Various studies done in SSA over the past decade have found different prevalence of albuminuria ranging from 10% in Ethiopia to 57% in Nigeria among patients with type 2 DM (Tesfaye, & Gill.). In our study, the prevalence of persistent albuminuria was found to be 18.5% among the patients. This is despite the short duration illness. Given that these are highly selected population of patients' newly diagnosed with type 2 DM, this figure is considered high.

Few studies have been done on the prevalence of persistent albuminuria. Similar to our study, Agarwal *et al.* in an Indian study found a prevalence of 17.3% among 300 patients diagnosed with type 2 DM within a period of six months (Agarwal et al., 2011). In contrasts, Agaba and others in a Nigerian study, found a higher prevalence of persistent albuminuria of 49.2% (Agaba et al., 2004). In the above two studies, urine albumin test was done on three different occasions of monthly intervals unlike in our study where the test was done on two different occasions 2-3 months apart. Hence, the variations in prevalence in these studies might be due to different population characteristics and study methodologies.

The global prevalence of albuminuria is reported to be higher (39%), among both type 1 and 2 DM patients and it included both spot and persistent albuminuria (Parving et al., 2006). This could be the reason for the higher prevalence of albuminuria.

We found the prevalence of spot albuminuria to be 29.4%. This compares well to the other previous Kenyan studies done in KNH which both found prevalence of spot albuminuria at 26% (Wanjohi et al., 2002: Mwendwa et al., 2005). The similarity is likely due to similar population characteristics in the KNH studies and our study. However, a USA study by Kohler and colleagues found a lower prevalence of spot albuminuria at 26.3% among African Americans using a lower cut off points (>25 mg/24hr) for albuminuria (Kohler et al., 2000). The lower prevalence in their findings might be due to better health seeking behavior in the developed countries leading to earlier diagnosis of DM.

Patients who had been diagnosed with type 2 DM within a period of less than three months were excluded due to the possibility of transient albuminuria as a result of hyperglycemia prior to the diagnosis of DM. We had a considerable number of patients who were on ACEI or ARBS. These drugs have been shown by several studies to retard the progression of diabetic nephropathy (Strippoli et al., 2005: Chawla et al., 2010: Ruggenenti et al., 2010: Fried et al., 2013). Despite this, it is unlikely to have affected the prevalence in our study as there was no difference between the two groups of patients with or without albuminuria. This might be due to the fact that higher doses are required in the treatment of albuminuria, and the patients might have been on these medications for a shorter duration of time.

In resource limited-settings, diagnosis of diabetic nephropathy is a challenge and is normally manifested by late presentation. However, there is limited data on duration of illness prior to diagnosis in SSA. Harris *et al*. in two population-based studies done in

Australia and United States showed that patients with type 2 DM have had the disease for an average of 4 to 7 years before diagnosis (Harris et al., 1992). It has been shown that untreated hyperglycemia initiates microvascular and macrovascular damage including diabetic nephropathy (Wardle, 1996). Therefore, the high prevalence of persistent albuminuria in our study may be a reflection of a longer interval between diabetes onset and diagnosis in this population. Poor health seeking behavior of the patients, limited resources and/or delayed diagnosis by clinicians could be some of the factors contributing to the late diagnosis in this population.

5.2 Patients' characteristics and associations with persistent albuminuria

5.2.1 Socio-demographic characteristics

There was a slight female predominance (58%) in our study. This could be a reflection of health seeking behavior among the sexes rather than higher incidences among female. This is supported by a study recently done in Nairobi slums that revealed that female patients are more likely to seek health care than their male counterparts (Muriithi, 2013). Wanjohi *et al.* in a KNH study done in 2002 also noted the same findings in his study where female constituted 67% (Wanjohi et al., 2002).

World Health Organization (WHO) report indicated that type 2 DM is common between the ages of 34 and 65 years in developing countries though it affects older population in developed countries (Beaglehole, 2004). This fact is in agreement with our study findings as majority of the patients were in their middle ages with a mean age of 55±12.5 years.

There was no significant association between age (p=0.126) or gender (p>0.33) as risk factors for albuminuria in this study. The KNH study by Wanjohi *et al*. also found similar findings (Wanjohi et al., 2002). However, those who had persistent albuminuria tend to be older (mean 59 years) and of male gender (53%). In contrast, a prospective Scottish study done in Edinburg by Patrick *et al.* and the Indian study done by Agarwal *et al*. showed increasing age and male gender as risk factors for albuminuria (Patrick et al., 1990: Agarwal et al., 2011). The differences with our study might be explained by differences in population characteristics.

Few patients (15%) reported history of cigarette smoking. This contrasts Wanjohi *et al*. KNH study that found that majority of the patients (73%) reported history of smoking (Wanjohi et al., 2002). The differences between these two studies could be under reporting by patients in our study and behavioral variations as KNH patients are mainly from urban areas. The above study and our study did not establish any significant association between history of smoking and albuminuria (p=0.143). This might be due to confounding effect of duration of smoking in our population compared to those in the west that are generally of older age and has longer duration of smoking (Meisinger et al., 2008: Keller et al., 1996).

5.2.2 Clinical and laboratory characteristics of the patients

In our study, the median duration of illness after the diagnosis was 9 months (IQR 3-14), which is comparable to two KNH studies which both found a mean duration of 10.3 (SD±7.5) months (Wanjohi et al., 2002: Mwendwa et al., 2005). In contrast, a study done by Kohler and colleagues in USA found a lower mean duration of 5 months (Kohler et al., 2000). The difference between our study and the above study might be due to the fact

that we excluded patients who had been diagnosed within a period of less than three months, who were included in their study.

Significant number of patients (63%) had a BMI above 25kg/m^2 and 62% had a high waist circumference (Female >88cm, Male >102cm). The KNH study, similarly found 66% of the study subjects being overweight (Mwendwa et al., 2005). This could be as a result of dietary changes and sedentary lifestyle among the population. However, the regression analysis showed no significant association between persistent albuminuria and BMI (p=0.0833). It is important to note that although these finding is similar to the KNH study (Wanjohi et al., 2002), a USA study (Kohler et al., 2000) done among 1,167 African Americans showed a significant association between albuminuria and BMI (p=0.0156). In addition, the NHANES III, Egyptian cross sectional study carried out between 1988 and 1994 that involved 5,659 participants, also showed a positive association between albuminuria and metabolic syndrome (P <.0001) (Palaniappan et al., 2003). Finally, Dustian *et al*. Australian diabetes and obesity study among 11,247 done in 2002, revealed significant association between metabolic syndrome and albuminuria (Dunstan et al., 2002). The variations between their findings and ours might due to the fact that the above studies had a larger sample size and also different patients' characteristics.

Despite the American Diabetes Association (ADA) recommended HbA1c target of less than 7%, majority of the patients (82.6%) in this study had an HbA1c above this range with a mean HbA1c of 10.1% (American Diabetes Associations, 2014). However, we didn't establish any significant association between hyperglycemia and persistent albuminuria (p=0.48). In contrasts, several studies have shown that hyperglycemia is

associated with albuminuria. Large randomized clinical trial studies such as; the DCCT (Worrall, 1994), ADVANCE (The ADVANCE Collaborative group, 2008), and ACCORD (Hoogwerf, 2008) all showed that tight blood glucose control has a protective role on the kidney. Furthermore, the Australian study also revealed that albuminuria worsens with worsening glycaemia (Dunstan et al., 2002). Since micro vascular and macro vascular complications are long term events, our study population could be at high risk due to poor glycemic controls.

The two previous KNH studies also revealed poor glycemic controls; Wanjohi *et al.* found 29% of the patients had good glycaemic control and Mwendwa *et al.* despite using a higher HbA1c cut-off of ≤8% found only 48% achieved this target (Mwendwa et al., 2005: Wanjohi et al., 2002). Nevertheless, a recent unpublished study done in MTRH in 2009 showed poor glycemic control among patients with type 2 DM with a mean HbA1c of 10.3±3.1% with 37% achieving good glycemic control using HbA1c of ≤7% (Faiz, 2006). There is no local study that has been done to investigate reasons for poor glycemic control in our setting, but it might be due to non compliance, poor patient education, denial, less stringent goals by clinicians among other factors.

In consistent with the two KNH studies which both found that 50% of the patients newly diagnosed with type 2 DM had hypertension, nearly half of the patients in our study (47.8%) had hypertension (Wanjohi et al., 2002: Mwendwa et al., 2005). However, in 2011 Wangari and others while studying all type 2 DM patients found a higher prevalence of hypertension (85%) in MTRH (Wangari, 2013). The higher prevalence in the above study is due to the fact that they used a lower cut off which was based on the JNC 7 guidelines (130/80mmHg) unlike the current JNC 8 cuts off (140/90mmHg) that

we used (Lenfant et al., 2003: James et al., 2014). Furthermore, our study was done among patients newly diagnosed with type 2 DM.

Most of the known hypertensive patients (79.5%) reported to be on antihypertensive medications with 56.8% (25/44) being on ACEI's or ARB's. A few, 10.4% (5/48) of patients reported no hypertensive history but were on ACEI's. It is important to note also that ACEI is not a first line recommended antihypertensive medications for patients with DM on the new JNC 8 guidelines (James et al., 2014). However, ACEI's/ARBs use among these patients could be explained by the fact that it recommended in the last JNC 7 guidelines as first line antihypertensive for patients with DM and hypertension at the time the study was conducted (Lenfant et al., 2003).

Poor adherence and health seeking behavior could be responsible for poor blood pressure control as a significant number (26%) of the patients had a high blood pressure (Bp ≥140/90) on the day of enrollment, and majority of those (55%) being known hypertensive.

We didn't establish any significant association between hypertension and persistent albuminuria in this study ($p>0.05$). However, we noted a higher proportion of hypertensive patients had persistent albuminuria. Many studies have showed that hypertension is a risk factor for albuminuria. Stratton *et al.* study revealed that hypertension and hyperglycemia have additive effects on risk of DM complications (Stratton et al., 2006). Similarly, Haneda *et al*. study among the Japanese patients noted high blood pressure was associated with risk of albuminuria (Haneda et al., 1992). Kohler and colleagues in the USA study also found that hypertension was significantly

associated with albuminuria ($p<0.0001$) among the African Americans (Kohler et al., 2000). In SSA, Agaba and others in a Nigerian study also found a positive association between albuminuria and hypertension (Agaba et al., 2004).

Dyslipidemia, a known risk factor for diabetic nephropathy, was also prevalent among patients (96%). This was shown by high prevalence of low HDL (82.6%) and high LDL (62%). The fact that only 8.7% of the patients were on statins could be attributed to low utilization of the screening services for dyslipidemia among the patients. It has been found that dyslipidemia is prevalent among diabetic patients and has a positive association with albuminuria (Mwendwa et al., 2005: Kohler et al., 2000: Agrawal et al., 2006).

Though majority of the patients with persistent albuminuria had high total cholesterol, high LDL, high triglycerides and low HDL, this did not reach a statistical significance. This is similar to Wanjohi *et al*. study findings which also did not establish any significant association between albuminuria and dyslipidemia (Wanjohi et al., 2002). However, it contrasts the Kohler *et al*. study which noted a strong association ($p=0.0003$) between dyslipidemia and albuminuria (Kohler et al., 2000). These variations might be due to different patients' characteristics.

Majority (93%) patients had eGFR above 60ml/min/1.73m^2 with a high mean of 104.9 (SD 33.4) ml/min/1.73m^2. It is widely known that early diabetic nephropathy is associated with hyperfiltration and high eGFR as first described in 1983 by Mogensen *et al.* (Mogensen et al., 1983). Hence, our patients with albuminuria including those without could be in this stage of diabetic nephropathy. We also found that 6 % of the patients had eGFR of less than 60 ml/min/1.73m^2. Several factors play a role in the

pathogenesis of diabetic nephropathy including interaction between genetic factors, various cytokines interaction with products of advanced glycation and oxidant stress (Dronavalli et al., 2008). Genetic testing was not done in this study to ascertain its role.

This study found positive association between persistent albuminuria and high serum creatinine (P=0.029) and low eGFR (P=0.016) (OR: 0.98 (95% CI: 0.96, 1.00). This is consistent with the study done by Kohler *et al.* in USA among African Americans which showed a significant association between albuminuria and serum creatinine level (P=0.0068) (Kohler et al., 2000). A meta-analysis that was done by Nitsch *et al*. (Nitsch et al., 2013) with over 2 million type 2 DM patients also revealed an increased risk of both cardiovascular and end stage renal disease with lower eGFR and albuminuria.

5.3 Summary of the key findings

In summary, there was a high prevalence (18.5%) of persistent albuminuria among this population, which is a marker of early diabetic nephropathy and increased cardiovascular morbidity and mortality (Henrichs, 1991: Gall et al., 1995). Known risk factors for albuminuria such as hypertension, hyperglycemia, and dyslipidemia were prevalent in this population. However, there was no significant association with these risk factors. The study established a significant association between persistent albuminuria and high serum creatinine and low eGFR. Therefore, clinicians should screen all patients with type 2 DM for albumunuria at diagnosis and intervene to retard the progression of albuminuria to ESRD.

5.4 Limitations of the study

1. This study being a cross-sectional study, could not establish causal inferences between persistent albuminuria and clinical, laboratory and socio-demographic characteristics .However, it does not affect the validity of the study.
2. Only one creatinine measurement was used to estimate eGFR instead of serial creatinine to rule out ongoing kidney injury. Nonetheless, it is unlikely to have affected the results as high eGFR was associated with persistent albuminuria reflecting chronic kidney damage.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. There was high prevalence (18.5%) of persistent albuminuria in patients newly diagnosed with type 2 diabetes at Moi Teaching and Referral Hospital.
2. There was a high prevalence of traditional risk factors for albuminuria such as; dyslipidemia, hyperglycemia and hypertension among the patients newly diagnosed with type 2 DM.
3. There was a significant positive association between high serum creatinine, low eGFR and persistent albuminuria

6.2 Recommendations

1. Screening for albuminuria, dyslipidemia and serum creatinine should be done at diagnosis of type 2 diabetes mellitus.
2. A prospective study should be done among the patients who had persistent albuminuria to determine their short term and long term outcomes.

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APPENDICES

APPENDIX I: Data collection form

Diabetes Type: 2

Biodata

IP.NO…….. Serial number……..

Age……..…years Gender……

Ethnicity……. Residence…….

Occupation……..…

Medical History

Duration of diabetes (months)…….

H/O Hypertension:…….Yes/No

H/O Stroke:……Yes/No

H/O Ischemic Heart Disease:……Yes/No

H/O Diabetic Retinopathy……..Yes/No

Family & social history

H/O Smoking:……Yes/No

H/O Diabetes in the family……..Yes/No

H/O kidney disease in the family…….Yes/No

Treatment history:

Glycaemic control agent (tick appropriate)

A. Insulin.....type ……

B. Oral hypoglycaemic agents; types: ……

C. Both Insulin and oral hypoglycaemics: types: …….

D. Diet control….

Use of ACE inhibitors/ARBs (tick appropriate)

A. Yes……name……..B No……

Lipid lowering agents (tick appropriate)

A. Yes……..name…….B No……

List other drugs:……

If female, LMP: **……**

EXAMINATION

General:

Pallor ☐ Jaundice ☐ Edema ☐

Dehydration ☐ Lymphadenopathy ☐

Height……..…cm **Weight:**……kg

BMI ……kg/m^2 **Waist Circumference**……cm

Vital signs:

BP: ……/……mmHg Pulse:

……../min

Temp: ……^oC $SPO_{2:}$ ……..%

Chest examination:

Normal ☐ Abnormal ☐

Heart examination:

Normal ☐ Abnormal ☐

Abdominal examination:

Normal ☐ Abnormal ☐

Nervous system examination:

Normal ☐ Abnormal ☐

Other findings

LABORATORY RESULTS

Laboratory test	**Results**	**Date**
FBS		
HBA1C		
Serum creatinine		
Total Cholesterol		
LDL		
HDL		
Triglycerides		
Urinalysis 1		
Urinalysis 2		
UACR 1		
UACR 2		

APPENDIX II: Consent form

English:

My name is Dr Joan Chepkorir Kiyeng. I am a qualified doctor, registered by the Kenya Medical Practitioners and Dentists Board. I am currently pursuing a Masters degree in Internal Medicine at Moi University. I would like to recruit you into my research which is to study if patients who newly diagnosed with type 2 diabetes mellitus could have a condition called diabetic nephropathy.

ABOUT DIABETIC NEPHROPATHY

A result from the effects of diabetes in the human body is kidney disease (DIABETIC NEPHROPATHY).

We shall screen you for other renal diseases, heart disease and any infectious illnesses that may present as fever and if you have any of those conditions you shall be excluded from the study although you will be appropriately offered treatment for your respective condition.

For us to know whether you have diabetic nephropathy, we will request you to undergo some tests preceding which you shall have to answer a few questions on your pertinent medical history. We will take a urine sample to check whether you are losing abnormal amounts of protein in your urine, and blood samples to check for average blood sugar for the last 2-3 months using HBA1c, we will also check for good and bad fats in your body using lipid profile .If you do not have protein in urine we will safely assume you are unlikely to have DIABETIC NEPHROPATHY. If you are losing abnormal amounts of protein in your urine, we will need to follow you and have a repeat check on your urine

within 2-3 months to ensure that you are actually losing abnormal amounts of protein in urine.

We will keep all your test results in confidence and keep you informed of the results. Treatment does not depend on your participation in this study. We will offer appropriate treatment for any condition that we find from assessing you and from your test results.

This study has been approved by the Institutional Research and Ethics Committee (IREC) of Moi University/Moi Teaching and Referral Hospital.

If you need further clarifications please contact IREC using the address below.

The Chairman IREC,
Moi Teaching and Referral Hospital,
PO Box 3,
Eldoret.
Tel: 33471/2/3
My cell phone number is: 0729172677

YOUR CONSENT:

Adults above 18 years of age

I have been adequately informed that I am being recruited in a study to find out if I have diabetic nephropathy. The investigator has also informed me that my participation in this study is voluntary and will not exclude me from my routine care even if I were to opt out. She has also informed me that I'll not be required to pay for the tests done for the purposes of this study.

Sign: ……

Name: ……

Date: ……

Kiswahili:

Jina langu ni Daktari Joan Chepkorir Kiyeng. Mimi ni daktari aliyefuzu nakusajiliwa na bodi ya madaktari wa Kenya (Kenya Medical Practitioners and Dentists Board). Mimi ni msomi wa shahada ya juu (Masters) ya udaktari (Internal medicine) katika chuo kikuu cha Moi University. Nimekuona leo kwa sababu ninafanya uchunguzi kujua kama watu wazima walionaugonjwa wa sukari huenda wakawa na ugonjwa wa figo utokanao ugonjwa wa sukari, ugonjwa huu kwa kiingereza unaitwa DIABETIC NEPHROPATHY.

KUHUSU NEPHROPATHY KISUKARI (DIABETIC NEPHROPATHY)

Moja ya madhara ya ugonjwa wa kisukari katika mwili wa binadamu ni ugonjwa wa figo (DIABETIC NEPHROPATHY). Ili kufanya uchunguzi huu, tutahakikisha kuwa huna magonjwa mengine ambayo yanajulikana kusababisha ugonjwa wa figo, tukipata kuwa unayo mojawapo wa magonjwa yoyote hayo, hutahitajika kushiriki katika uchunguzi huu, lakini utahudumiwa na kutibiwa ugonjwa huo vilivyo. Magonjwa hayo ni kama, ugonjwa wa roho. Tutahakikisha pia kuwa huna ugonjwa wowote usababishao homa au joto jingi mwilini. Tutakuuliza maswali kuhusu magonjwa yoyote mengine unayoweza kuwa nayo au unayotibiwa, historia ya jamii yako, madawa yoyote unayotumia na kisha tutakupima.

Ili tujue kama unayo ugonjwa huu wa DIABETIC NEPHROPATHY, tutakuomba tufanye uchunguzi kadhaa. hivyo tutapima *pressure* ya damu yako. Tutapima mkojo wako ndio tujue kama unamwaga protini kupita kiasi, tutahitaji tupime mkojo wako kwa mara nyingine tena katika muda usiopungua miezi miwili, ili tuwe na hakika kuwa ni kweli unapoteza protini kuliko kawaida. Siku utakaporudi tutapima mkojo wako tena, kama bado utakuwa unapoteza protini kupita kiasi, tutakushauri kuwa uko no ugonjwa wa figo,diabetic nephropathy,.Tutayaweka matokeo yako kwa njia ya kuheshimu haki

yako ya kutojulisha yeyote. Tutakujulisha kuhusu matokeo yako na maana kwa afya yako. Hatutakataa kupa matibabu iwapo utachagua ama usichague kushiriki katika uchunguzi huu.Tutakupa matibabu yafaayo kwa magonjwa yoyote tupatayo tukikuchunguza na yatakayopatikana kwa matokeo yako.

Uwe huru kuuliza maswali yoyote.Uchunguzi huu umehidhinishwa na kamati ya kusimamia machunguzi ya wasomi na haki ya wanaochunguzwa (Institutional Research and Ethics Committee-IREC) katika chuo kikuu cha Moi University na hospitali kuu ya Moi Teaching and Referral.

Iwapo unahitaji maelezo zaidi tafadhali wasiliana na IREC kwa kutumia anwani ifuatayo.

Mwenyekiti IREC,
Moi Teaching and Referral Hospital,S. L. P. 3,
Eldoret.
Simu: 33471/2/3
Nambari yangu ya simu ya rununu ni: 0729172677

IDHINI YAKO:

Walio na miaka 18 na zaidi

Nimeelezwa ipasavyo ya kwamba ninashiriki katika uchunguzi wa usomi utakayo chunguza iwapo ninaugua ugonjwa wa figo utokanao na ugonjwa wa kisukari, ugonjwa ambao mimi kwa ajili ya kuwa na ugonjwa wa sukari ninaweza kuwa nayo. Mchunguzi pia amenieleza kuwa sitakosa matibabu yangu ya kawaida iwapo nishiriki katika uchunguzi au nisiposhiriki. Pia nimeelezwa kuwa sitahitajika kulipia chochote kinachohusiana na uchunguzi huu.

Sahihi: ……
Jina: ……
Tarehe:……

APPENDIX III: Procedure for measuring blood pressure

Blood pressure will be taken using an Omron M2 compact upper arm blood pressure monitor (**Omron Healthcare, Inc., 1200 Lakeside Drive, Bannockburn, Illinois 60015**).

The patient should be in a quiet place, in a relaxed sitting position with no tight fitting clothing on the upper arm, or any thick clothing such as a sweater.

The patient sits upright with the back straight and places the arm on the table so that the cuff is on the same level as the heart. The cuff is wrapped on the right arm such that the bottom of the cuff is at least 1cm above the elbow. It is then fastened snugly. The start button on the machine is then pressed and automatically the cuff begins to inflate and the machine takes a reading. The blood pressure results as well as a heart rate reading are then displayed on the screen.

Should an error occur, the cuff is deflated and the process is repeated. High blood pressure readings are confirmed manually using a mercury sphygmomanometer.

The blood pressure machines are calibrated every week.

APPENDIX IV: Procedure for testing blood glucose

Instrument used: HemoCue Glucose 201+

Principles:

The HemoCue Glucose 201+ is a system for the determination of the total amount of glucose in whole blood. Capillary, venous or arterial blood may be used. It utilises a modified glucose dehydrogenase method. A chromogen compound is added to the reagents with saponin used for haemolysing the erythrocytes. The absorbance is measured at two wavelengths (660 and 840nm) to compensate for turbidity.

Testing Procedure: Procedure is explained to the subject and consent obtained. Subject's middle finger is swabbed with methylated spirit and allowed to dry. Using the technician's thumb, the finger is pressed from the top of the knuckle towards the tip to stimulate blood flow. Whilst lightly pressing towards the finger tip, the swabbed finger is pricked. The first drop of blood is wiped and light pressure towards the finger tip replied until another drop of blood appears. A new microcuvette is removed from its pack. A drop of blood is placed at the tip of the microcuvette allowing it to fill in a continuous process making sure that there are no air bubbles. Wipe off excess blood on the outside of the microcuvette tip without drawing blood out of the cuvette. If there are air bubbles, the test is repeated with a new sample of blood. Place the filled microcuvette in the cuvette holder within 40 seconds of filling the cuvette. Push the cuvette holder to its measuring position. Test results are automatically displayed in 40-240 seconds.

Quality Control

The HemoCue Glucose 201+ analyser has an internal electronic „SELFTEST". Every time the analyser is turned on, it will automatically verify the performance of the optronic unit of the analyser. This test is performed every second hour if the analyser is left turned on.

Measuring range:

0-30mmol/L (0-540mg/dL). Results above 30mmol/L (540mg/dL) will be displayed as HHH.

IVD Medical Device Directive:

The HemoCue Glucose 201+ complies with the IVD Medical Device Directive 98/79/EC and carries the CE mark. 61

APPENDIX V: Procedure for HbA1c Assays

HbA1c Analyzer used: Bayer DCA 2000®+Analyzer

Chemical Principles of procedure:

Both the concentration of haemoglobin A1c and concentration of total haemoglobin are measured and the ratio reported as percentage HbA1c. All of the reagents for performing both reactions are contained in DCA 2000® HbA1c reagent cartridge. For the measurement of total haemoglobin potassium ferricyanide is used to oxidize haemoglobin in the sample to methaemoglobin. The methaemoglobin then complexes with thiocyanate to form thiocyanomethaemoglobin, the coloured species which is measured. The extent of colour development at 53-nm is proportional to the concentration of total haemoglobin in the sample. For the measurement of specific HbA1c, an inhibition of latex agglutination assay is used. An agglutinator causes agglutination of latex coated with HbA1c specific mouse monoclonal antibody. This is measured as an increase in absorbance at 531nm.HbA1c competes for the limited number of antibody-latex binding sites causing an inhibition of agglutination and a decreased scattering of light. The decreased scattering is measured as a decreased in absorbance at 531nm.The HbA1c concentration is then quantified using a calibration curve of absorbance versus HbA1c concentration. All measurements and calculations are calculations are performed automatically by the DCA 2000+ Analyzer, and the screen displays percent HbA1c at the end of assay.

Procedure for HbA1c Assay; Procedure is explained to the subject and consent obtained. Subject's finger is swabbed with methylated spirit and allowed a few seconds to dry. The

swabbed Finger pricked. A new plastic capillary holder is removed from a blister pack. A drop of blood is placed at the end of glass capillary allowing the glass capillary to fill. A new reagent cartridge is opened up. The plastic capillary holder is placed into the reagent cartridge. The reagent cartridge holder is loaded into the DCA 2000® +Analyzer. Test results are automatically displayed in 6 minutes.

Calibration:

Instrument: The DCA 2000+Analyzer is calibrated by the manufacturer. Thereafter, the instrument automatically self-adjusts during first-time power up and during each assay. In the event of the system is unable to make appropriate internal adjustments, an error message is displayed.

Reagent:

The manufacturer calibrates the cartridges using parameters based on a DCCT reference method. The DCA 2000 HbA1c test method is National Glycohaemoglobin Standardization Program (NGSP) certified. The DCA 2000 HbA1c test method is traceable to International Federation of Clinical Chemistry (IFCC) reference materials and test methods. There is a calibration card for scanning for each lot of cartridges; the calibration bar code is read by the instrument. This assesses the appropriate calibration values for the particular lot. If no calibration curve is in use, the instrument prompts the user to scan the calibration card. 63

Quality Control:

To assure quality of both testing procedures and patient results for haemoglobin A1c, the DCA 2000+ system performs 48 optical, electronic, mechanical and reagent checks during the course of each specimen assay.

In addition to the above measures, each new lot had one kit used for assessing quality control by checking the HbA1c percent of healthy non diabetic persons.

Blood in plain Vacutainer® bottles are taken immediately to the lab. Serum may be stored for up to one day at 2 to 25ºC, up to seven days at 4 to 8ºC and up to six months at -20 to -80ºC.

The bottle is set onto a centrifuge and spun at 3000 rpm for 3 minutes to separate the serum from the cells. The supernatant (serum) is carefully suctioned using a micropipette and transferred to a sample cup.

The sample cups are systematically set on a rack that goes onto a Cobas Integra® 400 plus analyzer (**Roche Diagnostics, 9115 Hague Road, PO Box 50457, Indianapolis, IN 46250-0457**). This is an autoanalyzer that uses the Jaffe reaction to quantify creatinine; creatinine reacts with picric acid in the presence of an alkaline pH to produce a yellow-red complex that has a maximum absorbance at 512nm. The rate of dye formation is proportional to the level of creatinine in the sample. The analyzer reads out this absorbance and based on its software it calculates the serum creatinine. It prints out the result on paper.

The result is reported in µmol/L alongside reference serum creatinine levels.

Quality control checks are run daily.

APPENDIX VI: Procedure for drawing blood

The procedure is explained to the patient and verbal consent sought.

Universal precautions will be observed.

A tourniquet is applied at a distal site about 5cm proximal to the selected site of venepuncture. The patient makes a fist without pumping the hand. The phlebotomist puts on a pair of clean gloves. The selected site is cleaned thoroughly with methylated spirit or povidone Iodine starting with the center and working outward. It is then allowed to dry.

The patient's arm is grasped firmly using the thumb to keep the skin taut and to anchor the vein. A sterile Vacutainer® system (**Becton, Dickinson and Company, 1 Becton Drive, Franklin Lakes, NJ USA 07417**) is opened and the blood collection needle inserted gently into the lumen of the vein at an angle of 15- 30^{o}, then the other end is attached to a Vacutainer® blood collection bottle. Blood flows freely into the bottle due to negative pressure. 2ml of blood for serum creatinine determination will be collected in a plain bottle and another 2ml will be collected in a S.S.T-bottle to be used for determination of the lipid profile.

After adequate blood has been collected, the tourniquet is released then the Vacutainer® needle is removed gently and an alcohol impregnated swab is applied at the site under pressure. Pressure is applied for a whole minute then the site is reassessed for continued bleeding. The area is dressed with a dry gauze and tape.

APPENDIX VII: Procedure for measuring lipid profile

HDL CHOLESTEROL liquicolor is a homogenous enzymatic assay for the quantitative determination of HDL cholesterol. Blood is drawn after an overnight fast.

Method

The assay combines two specific steps: 1st step chylomicrons, VLDL and LDL cholesterol are specifically eliminated and destroyed by enzymatic reactions. In the second step remaining cholesterol from HDL fraction is determined by well established specific enzymatic reactions in the specific surfactants for HDL.

CHOLESTEROL liquicolor CHOD-PAP-Method Enzymatic Colometric Test for cholesterol with Lipid Clearing Factor (LCF)

Method

The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminophenazone in the presence of phenol and peroxidase.

TRIGLYCERIDES liquicolor GPO-PAP Method Enzymatic Colometric Test for Triglyceridess with Lipid clearing Factor (LCF)

Method

The triglycerides are determined after enzymatic hydrolysis with lipases.Indicator is quinoneiminine formed fromhydrogen peroxide, 4-amino-antipyrine and 4-chlorophenol under the catalytic influence of peroxidase.

Quality control

For all this quality control are run daily

APPENDIX VIII: Procedure for determining serum creatinine

Blood in plain Vacutainer® bottles are taken immediately to the lab. Serum may be stored for up to one day at 2 to 25^{o}C, up to seven days at 4 to 8^{o}C and up to six months at -20 to -80^{o}C.

The bottle is set onto a centrifuge and spun at 3000 rpm for 3 minutes to separate the serum from the cells. The supernatant (serum) is carefully suctioned using a micropipette and transferred to a sample cup.

The sample cups are systematically set on a rack that goes onto a Cobas Integra® 400 plus analyzer (**Roche Diagnostics, 9115 Hague Road, PO Box 50457, Indianapolis, IN 46250-0457**). This is an autoanalyzer that uses the Jaffe reaction to quantify creatinine; creatinine reacts with picric acid in the presence of an alkaline pH to produce a yellow-red complex that has a maximum absorbance at 512nm. The rate of dye formation is proportional to the level of creatinine in the sample. The analyzer reads out this absorbance and based on its software it calculates the serum creatinine. It prints out the result on paper.

The result is reported in µmol/L alongside reference serum creatinine levels.

Quality control checks are run daily.

APPENDIX IX: Procedure for urinalysis

Fresh urine is collected from a patient using a clean, dry bottle and split into two bottles.

1. **Dipstick urinalysis**

   A Uristix® strip (**Siemens Healthcare Diagnostics, 1717 Deerfield Road, Deerfield, IL 60015-0778, USA**) is briefly immersed in the urine specimen, covering all reagent areas.

   The edge of the Uristix® strip is run against the rim of the urine container to remove excess urine. The strip is held in a horizontal position.

   The strip measures proteinuria based on 'protein error of pH indicators' principle. This principle states that at constant pH the development of any green color is due to the presence of protein. The result ranges from yellow for negative results to yellow green to green blue for positives. The test pad contains a pH dye indicator using bromphenol blue. Due to the negative charge of albumin, if protein (albumin) is present in urine, the pH increases, and a positive test result occurs.

   The reactions are read visually. The strip test area is compared to that on the Uristix® color chart. This will be read after 60 seconds. The color at the center of the pad is compared to the corresponding color chart on the bottle label. 2 observers will read the color and a third person acts as a tie-breaker if there is disagreement.

   The results are recorded, and the strip is discarded.

   Normal and abnormal controls will be run daily to ensure validity of results.

2. **Urinary albumin to creatinine ratio (UACR)**

   Fresh urine is taken immediately after collection to the lab for assay.

Urine is assayed for the level of albumin and creatinine in the lab using the Cobas Integra® 400 analyzer (**Roche Diagnostics, 9115 Hague Road, PO Box 50457, Indianapolis, IN 46250-0457**).

This is an autoanalyzer that uses the Jaffe reaction to quantify creatinine; creatinine reacts with picric acid in the presence of an alkaline pH to produce a yellow-red complex that has a maximum absorbance at 512nm. The analyzer reads out this absorbance and based on its software it calculates the urine creatinine.

It quantifies urinary protein by colorimetric method. Pyrogallol-red forms a complex with molybdate that is red in color with a maximum absorbance at 470nm. Binding of basic amino acids of proteins to this pyrogallol-red molybdate complex induces a spectral shift forming a blue-purple complex. The color intensity of this blue-purple complex is directly proportional to the level of proteins in the urine. The analyzer using software calculates this. It prints out the result on paper.

The results are reported in mg/dl for both creatinine and albumin.

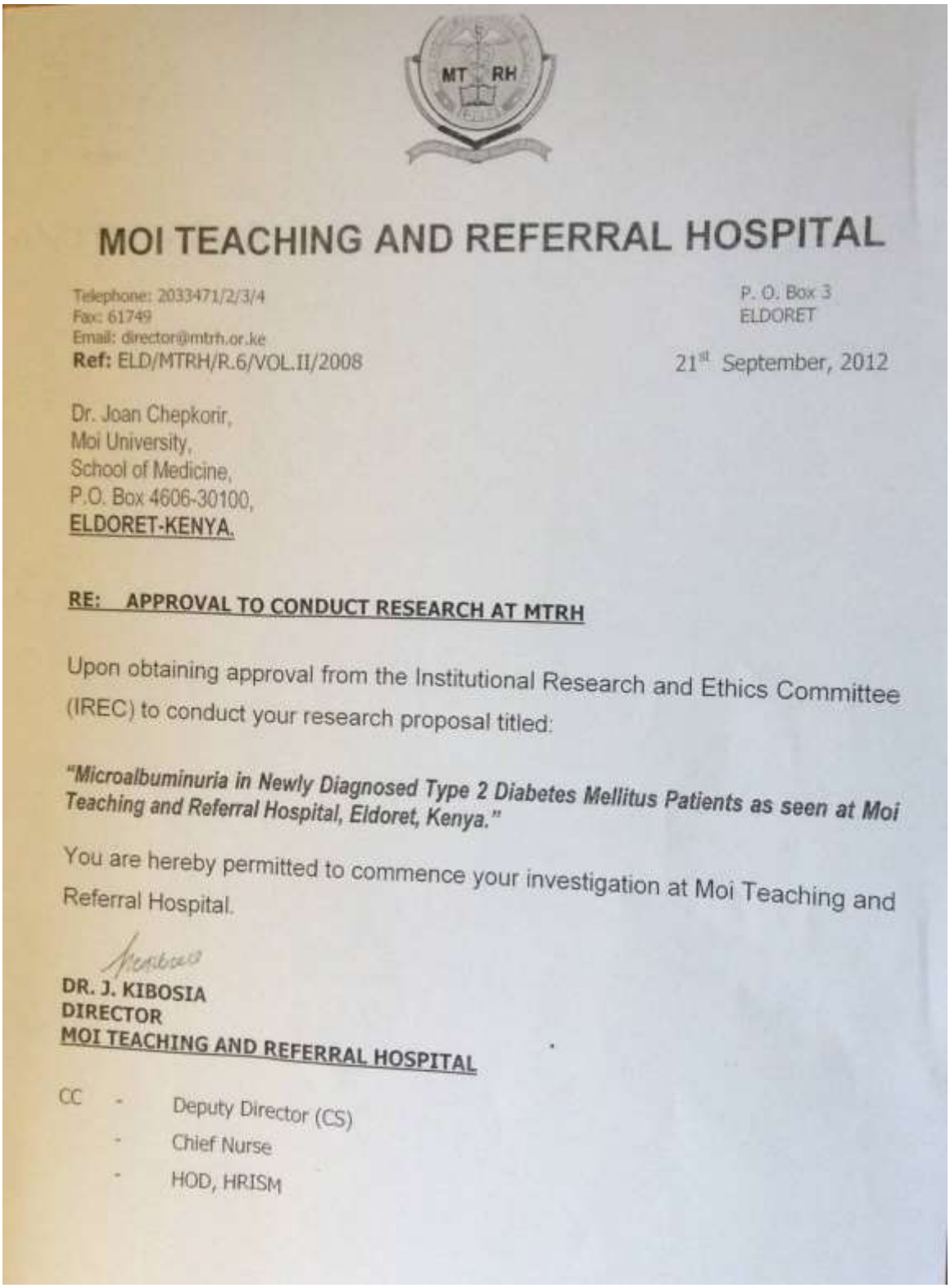
Quality control checks are run daily.

The UACR is calculated thus:

$$UACR = \frac{Albumin\,(mg/dl)}{Creatinine(g/dl)}$$

The result from the above equation gives a fairly accurate estimate of the 24 hour albumin excretion in mg/day; this can be converted to g/day by dividing by 1000.Normally there should be less than 30mg of albumin excreted per day, a value between 30 and 300mg/day is not detectable by dip stick and is called microalbuminuria, beyond 300 mg/day dipsticks detect it and is called macroalbuminuria (proteinuria).

APPENDIX X: MTRH Approval



MOI TEACHING AND REFERRAL HOSPITAL

Telephone: 2033471/2/3/4
Fax: 61749
Email: director@mtrh.or.ke
Ref: ELD/MTRH/R.6/VOL.II/2008

P. O. Box 3
ELDORET

21st September, 2012

Dr. Joan Chepkorir,
Moi University,
School of Medicine,
P.O. Box 4606-30100,
ELDORET-KENYA.

RE: APPROVAL TO CONDUCT RESEARCH AT MTRH

Upon obtaining approval from the Institutional Research and Ethics Committee (IREC) to conduct your research proposal titled:

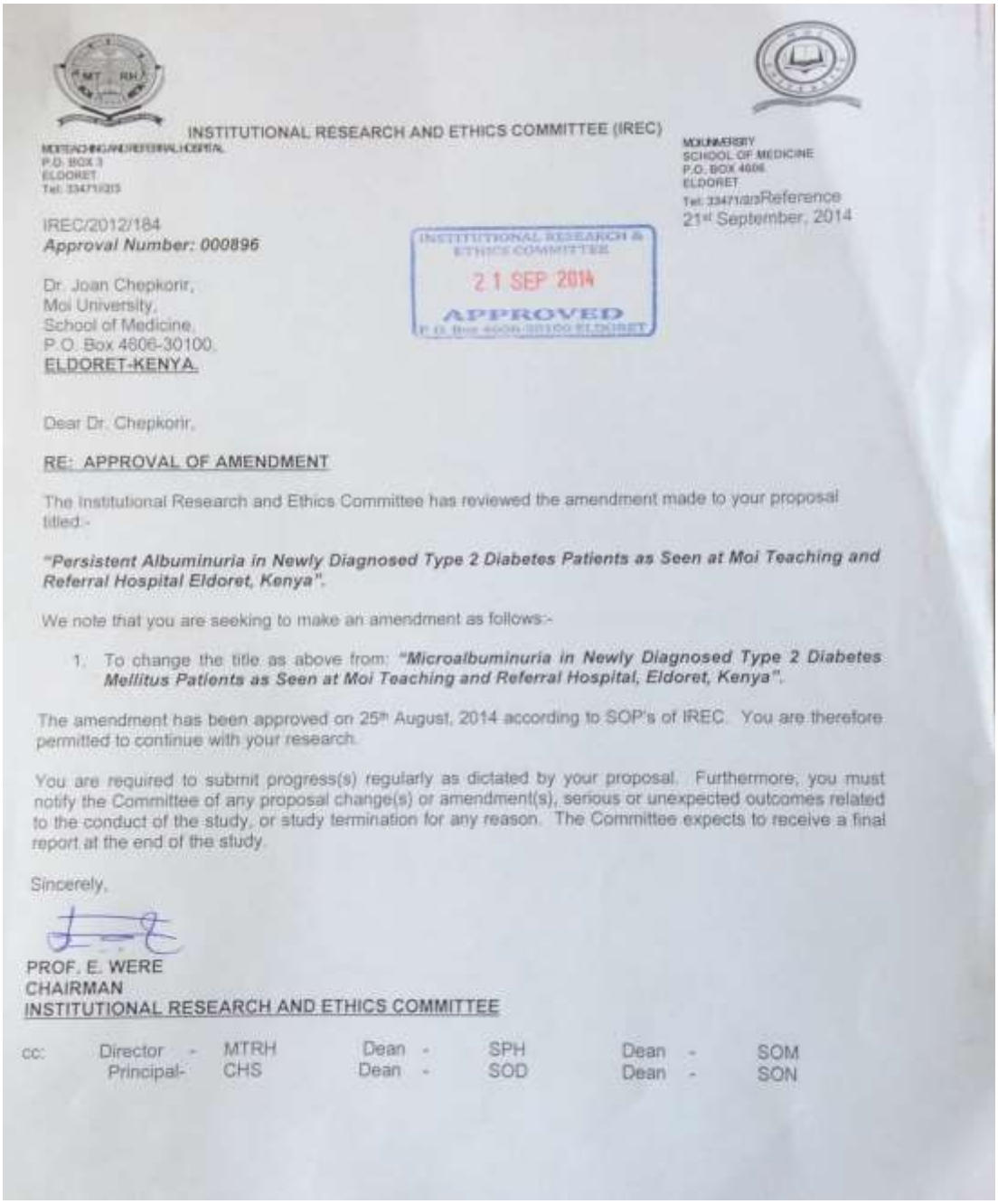
"Microalbuminuria in Newly Diagnosed Type 2 Diabetes Mellitus Patients as seen at Moi Teaching and Referral Hospital, Eldoret, Kenya."

You are hereby permitted to commence your investigation at Moi Teaching and Referral Hospital.

DR. J. KIBOSIA
DIRECTOR
MOI TEACHING AND REFERRAL HOSPITAL

CC - Deputy Director (CS)
- Chief Nurse
- HOD, HRISM

APPENDIX XI: IREC approval



INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471/2/3

MOI UNIVERSITY
SCHOOL OF MEDICINE
P.O. BOX 4606
ELDORET
Tel: 33471/2/3

IREC/2012/184
Approval Number: 000896

Reference
21st September, 2014

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
21 SEP 2014
APPROVED
P.O. Box 4606-30100 ELDORET

Dr. Joan Chepkorir,
Moi University,
School of Medicine,
P.O. Box 4606-30100,
ELDORET-KENYA.

Dear Dr. Chepkorir,

RE: APPROVAL OF AMENDMENT

The Institutional Research and Ethics Committee has reviewed the amendment made to your proposal titled:-

"Persistent Albuminuria in Newly Diagnosed Type 2 Diabetes Patients as Seen at Moi Teaching and Referral Hospital Eldoret, Kenya".

We note that you are seeking to make an amendment as follows:-

1. To change the title as above from: ***"Microalbuminuria in Newly Diagnosed Type 2 Diabetes Mellitus Patients as Seen at Moi Teaching and Referral Hospital, Eldoret, Kenya".***

The amendment has been approved on 25th August, 2014 according to SOP's of IREC. You are therefore permitted to continue with your research.

You are required to submit progress(s) regularly as dictated by your proposal. Furthermore, you must notify the Committee of any proposal change(s) or amendment(s), serious or unexpected outcomes related to the conduct of the study, or study termination for any reason. The Committee expects to receive a final report at the end of the study.

Sincerely,

PROF. E. WERE
CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc: Director - MTRH Dean - SPH Dean - SOM
Principal- CHS Dean - SOD Dean - SON