EFFECT OF HYPERTENSION ON RENAL FUNCTIONS IN DIABETICS ATTENDING A REFERRAL HOSPITAL IN KENYA

By

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Declaration

Declaration by the Candidate

This is my original work and to the best of my knowledge has not been presented for any degree in any other university. No part of this work can be produced without the prior permission of the author and/ University of Eldoret.

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Declaration by Supervisors

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

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Abstract

Hypertension affects and progressively reduces the filtering function of the kidney leading to chronic kidney disease. There are many incidences of people with high blood pressure hence chronic kidney disease is expected to be common. Chronic kidney disease is diagnosed by renal function tests in the serum and urine. Diabetes also causes chronic kidney disease and is fairly common hence coexistence with hypertension has synergistic insult on the kidney. The prevalence of hypertension in Kenya is approximated to be 44.5% among the male population and 35.1% among the female population. On the other hand, the prevalence of diabetes is 10% and is reported to be independent of age and gender distributions. Information about the extent of kidney disease among hypertensive diabetics in our setting is lacking therefore there is need for studies to fill this gap. The objective of the study was to establish whether hypertension hightens kidney dysfunction in diabetic patients. The study was cross-sectional and was carried out at Moi Teaching and Referral Hospital, Eldoret, Kenya between December 2011 and April 2012. Three hundred and twenty four (324) hypertensive diabetics and non-hypertensive diabetics meeting the inclusion criteria were consecutively selected and requested for consent before their recruitment into the study. Serum and urine samples were obtained from the patients and analysed using Diacetylmonoxime, Jaffe's, Sulphosalicyclic acid and Urinometer methods to obtain creatinine, urea, protein and glucose levels respectively. . These data together with demographic characteristics including age, sex and weight obtained from the hospital records were entered into the Computer using SPSS version 17 (Levesque, 2007). Analysis consisted of descriptive statistics for the variables and quantitative correlation between the hypertensive and non hypertensive diabetics at 95% confidence level using Chi-square. Significant difference in serum urea was observed between the two study groups with hypertensive diabetics having a mean of 57.8 mg/dl while the non-hypertensive group had a mean of 17.3 mg/dl. Hypertensive study group had significantly higher creatine levels compared to that of non-hypertensive control group (1.55 mg/dl/2.43mg/dl, P= 0.05). On the other hand, 19.24 mg/dl and 15.3 mg/dl urea levels were observed in the Hypertensive and non-hypertensive study groups respectively. Although not significant, the study observed a general increasing trend of all the serum and urine study parameters from the non-hypertensive's to pre-hypertensive with highest levels being observed in the high blood pressure subjects.

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List of Abbreviations

IDF- International Diabetic Federation

IDDM-insulin-dependent diabetes

NIDDM-non-insulin-dependent diabetes mellitus

IDDM-insulin dependent diabetes

CVD- cardiovascular disease

HDL - high density lipoprotein

NHANES -National Health and Nutrition Examination Survey

BUN- blood and urine urea nitrogen

ESRD- end-stage renal disease

GN- glomerulonephritis

ACR- albumin/creatinine ratio

MTRH-Moi Teaching and Referral Hospital

BP-Blood pressure

CHAPTER ONE

1.0. INTRODUCTION

1.1. Background

Kidney disease is a common and serious progressive complication of diabetes mellitus which has a major impact on patient morbidity and mortality and therefore a profound impact on the delivery of health care (Todd, 2008). It is a disease with universal distribution affecting all populations (Saunders, 1996). Hypertension affects more than one third of patients with insulin-dependent diabetes mellitus (type 1 diabetes), and up to a quarter of all patients with non-insulin-dependent diabetes mellitus (type 2 diabetes) and therefore it is the single most common cause of end-stage renal failure in western countries and its incidence continues to rise (National Heart Foundation of Australia, 2008).There are currently more than 240 million people with diabetes worldwide and the top five countries with the highest prevalence of diabetes from highest to lowest include India, China, the United States, Russia, and Japan. This figure is projected to rise to 380 million by 2025, largely as a result of population growth, aging, urbanization, unhealthy eating habits, increased body fat, and a sedentary lifestyle. By 2025, the number of people with diabetes is expected to more than double in Southeast Asia, the Eastern Mediterranean and Middle East, and Africa. It is projected to rise by nearly 20% in Europe, 50% in North America, 85% in South and Central America, and 75% in the Western Pacific region (Alwakeel *et al.*, 2009; Bakris and Ritz, 2009). In Kenya, the Ministry of Health estimates the prevalence of diabetes to be around 10% (3.5 million people) and its expected to rise causing much human suffering and considerable

economic burden on individuals, families, and healthcare systems (Atieno, 2006; Zhuo *et al*., 2013).

In diabetics, kidney disease first manifests with persistent microalbuminuria, defined as albumin excretion ranging from 20–200 mg/24 hr. Subsequently, kidney disease is heralded by the appearance of persistent proteinuria (defined as a total protein excretion of >200 mg/day) associated with the early onset of hypertension. Following the onset of proteinuria, there is a progressive decline in glomerular filtration rate which results in end-stage renal disease in 50% of those with nephropathy after about 5 years (Brenner and Rector's, 2000 ; Saud *et al*., 2009).

Hypertension and diabetes are common diseases that coexist (Berraho *et al*., 2013) and are now regarded as a major health threat in Africa where according to International Diabetic Federation (IDF, 2012) about 10 million people now have both conditions. This constitutes about 3.1% of adult population and is estimated that this will almost double in the next 15 years (Ritz and Orth, 1999a; Atieno, 2006). Hypertension interacts with diabetes to cause microvascular disease of the kidneys resulting in impairment of wastes and extra fluid removal from the body. This extra fluid in the blood vessels may result in a further rise of blood pressure (Diabetes Care, 2005). In type 1 diabetes, hypertension may reflect the onset of diabetic nephropathy and in type 2 diabetes, hypertension is often present as part of the metabolic syndrome of insulin resistance also including central obesity and dyslipidemia (Alwakeel *et al*., 2009).

1.2. Significance of the study

Studies on comparative renal function among hypertensive diabetics and non-hypertensive diabetics are few. Because of the aging population, lifestyle changes, and consequent increasing prevalence of hypertension and diabetes seen in our setting, chronic kidney disease will continue to increase placing an undue economic burden on societies, given the costs for an end stage kidney disease program. Knowing the renal functions in both groups can be useful in guiding clinicians and other health care providers whether testing for renal function is cost effective in the management of chronic kidney disease and to give information about the use of the reno-protective antihypertensive drugs.

1.4. Objectives

1.4.1. General objective

To determine the levels of kidney dysfunction between hypertensive diabetics and non-hypertensive diabetics at MTRH in Kenya.

1.4.2. Specific objectives

1. To determine the serum creatinine, urea, proteins and glucose levels in hypertensive diabetics and non-hypertensive diabetics.
2. To determine the urine creatinine, urea, proteins and glucose levels in hypertensive diabetics and non-hypertensive diabetics.
3. To compare serum creatinine, urea, proteins and glucose levels in various degrees of hypertension in hypertensive diabetics.

1.3. Research Hypothesis

H_o: There is a difference on the integrity of kidneys between diabetics with hypertension and those without as measured by serum and urine creatinine, urea, proteins and glucose levels.

CHAPTER TWO

2.0. LITERATURE REVIEW

Hypertension, commonly referred to as "high blood pressure" is a medical condition in which the blood pressure is chronically elevated where systolic blood pressure is consistently 140 mmHg or greater, and diastolic blood pressure is consistently 90 mmHg or greater. Hypertension is classified as either essential (primary) or secondary. Essential hypertension indicates that no specific medical cause can be found to explain a patient's condition. Secondary hypertension indicates that the high blood pressure is a result of (i.e. secondary to) another condition, such as kidney, diabetes or certain tumors (especially of the adrenal gland) ((EGIR), 2002; WHO, 2006). Diabetes mellitus describes a medical disorder of multiple etiology characterized by chronic hyperglycemia with disturbance of carbohydrate, fat and protein metabolism resulting from the beta cell loss function of varying degrees (Souhami and Moxham, 2006; WHO, 2006; Nicholas et al., 2006). It is characterized as absolute (type 1) or relative (type 2) insulin insufficiency and approximately 10% of diabetic patients have type 1 while 90% have Type 2. The effects of the disorder include long-term damage, dysfunction and failure of various organs.

Renal failure or kidney failure describes a medical condition in which the kidneys fail to adequately filter toxins and waste products from the blood. The kidneys undergo cellular death and are unable to filter wastes, produce urine, and maintain fluid balances. Renal failure can be divided into two categories: acute renal failure or chronic renal disease. Acute kidney failure usually occurs when the blood supply to the kidneys is suddenly

interrupted or when the kidneys become overloaded with toxins (Jafar, 2003). Chronic renal failure is more serious than acute renal failure because symptoms may not appear until the kidneys are extremely damaged and possible causes are diabetes mellitus and high blood pressure. Others include glomerulonephritis caused by post infectious condition and lupus, use of analgesics such as acetaminophen and ibuprofen regularly over a long duration, clogging and hardening of the arteries leading to the kidney, obstruction of the flow of urine by the stones, strictures or cancers. Chronic renal failure can worsen over time, especially when the problem has gone undiagnosed and treatment is delayed (Sowers *et al*., 2001).

2.1. Hypertension in Diabetics

Hypertension and diabetes mellitus especially type 1 insulin-dependent diabetes (IDDM) share the same risk factors that include excessive body mass, unhealthy diets, tobacco use, hereditary and physical inactivity level. Because they both occur together so frequently they are considered to be comorbidities where diabetes makes high blood pressure more difficult to treat, and high blood pressure makes diabetes even more dangerous.(Sowers *et al*., 2001).

Hypertension and diabetes are likely to occur together in one person (Berraho *et al*., 2013) and tend to get worse with time; however, diabetics can get hypertension as a complication secondary to renal damage. The self-reinforcing relationship between two conditions takes place in the kidneys-the long-term blood pressure regulator. By balancing the amount of salt and potassium in the body, the kidneys ultimately control how much fluid is excreted as urine. This fluid regulating function helps modulate long-

term blood pressure by physically controlling how much liquid is present in the blood vessels. Carrying out this filtering function depends on a constant flow of blood across delicate capillary structures the glomeruli.

The high blood sugar levels associated with diabetes damage glomeruli capillaries through a complex series of steps, where excess blood sugar actually causes its thickening and the degradation hence do not receive enough blood. As a result the kidneys respond by raising intrarenal blood pressure to restore the normal blood flow through the glomeruli. Because they have been damaged, the glomeruli essentially require a permanent increase in blood pressure in order to continue filtering the blood. Continued exposure to elevated sugar damages the glomeruli more, leading to persistent ever increasing intrarenal blood pressures as the kidneys try to correct the situation (Jafar, 2003).

Persistent elevated intrarenal hypertension has widespread effects on the other organ systems of the body. In the muscles, higher pressure causes blood vessels to contract resulting into less blood flowing through the large muscle areas of the body. This leads to a decrease in the size of muscle cells and a decrease in the amount of sugar that those cells absorb from the blood. Because less sugar is being absorbed from the blood, the level of free sugar in the blood rises. This free sugar makes its way to the kidneys, where it contributes to further glomerular damage accelerating the renal complications. Altered blood flow through the pancreas, as a result of autoregulation, can also lead to a decrease in insulin production, raising the blood sugar even higher (Sowers *et al.*, 2001; Jafar, 2003).

Over the last decade, there has been increasing interest in the clinical association between diabetes and hypertension because they share certain synergistic physiological traits. These physiological traits may include one or more of the following; diabetes increases the total amount of fluid in the body, which tends to raise blood pressure, diabetes decreases the ability of the blood vessels to stretch, increasing average blood pressure and diabetes changes the production and handling of insulin in the body causing an increase in blood pressure (EGIR, 2002; Alwakeel *et a*l., 2009).

Research has shown that hypertension is twice more prevalent in diabetic than in non-diabetic individual (EGIR), 2002). Furthermore, it has been clearly shown that hypertension in diabetic patients is associated with accelerated (Berraho *et al*., 2013) progression of both microvascular and macrovascular complications (EGIR, 2002). Macrovascular disease accounts for the majority of deaths in patients with non-insulin-dependent diabetes mellitus (NIDDM). The presence of hypertension in this type of diabetes is associated with a 4-5-fold increase in mortality, predominantly from coronary artery disease and stroke (WHO, 2006). In addition, insulin dependent diabetes (IDDM) accounts for majority of nephropathy which aggravates both macro and microvascular complications of diabetes mellitus (Alwakeel *et al*., 2009). Another research study has shown that almost 75% type 2 diabetic patients with kidney problems had high blood pressure while 40% of diabetic patients with no kidney problems had high blood pressure. Overall, when averaged across diabetes type and age range, about 35% of all people with diabetes have high blood pressure (National Kidney Foundation, 2008).

NIDDM and hypertension commonly co-exist and may be part of the insulin resistance or metabolic syndrome. Insulin resistance is considered the main threat to public health in

the 21st century and is associated with an increased risk of cardiovascular disease (CVD) (Taskinen, 2007) and the clinical consequences of insulin resistance include dyslipidemia (Ginsberg, 2000) hyperglycemia, hypertension and abnormal vascular behavior, vascular inflammation and risk of thrombotic inflammation (Gustafson *et al*., 2007). NIDDM describes a group of clinical and biochemical features, which are strongly associated with, accelerated atherosclerosis. These features include obesity, mixed dys-lipidaemia (high triglycerides, low high density lipoprotein and cholesterol levels), hyperinsulinaemia, and hypertension. The underlying association between hypertension and diabetes in this syndrome remains unknown, but it is possible that endothelial dysfunction as a result of both hypertension and diabetes could be an important factor in the high incidence of vascular disease in individuals with both conditions (EGIR, 2002).

Insulin is a polypeptide hormone secreted by the pancreas and its main purpose is to regulate the levels of glucose in the body antagonistically with glucagon through negative feedback loops and also exhibits vasodilatory properties (Stuart, 2004). In normotensive individuals, insulin may stimulate sympathetic activity without elevating mean arterial pressure. However, in more extreme conditions such as that of the metabolic syndrome, the increased sympathetic neural activity may over-ride the vasodilatory effects of insulin. Insulin resistance and/or hyperinsulineamia have been proposed to be responsible for the increased arterial pressure in some patients with hypertension (Diabetes Care, 2005).

2.2. Hypertension and Renal function in Diabetics

High blood pressure is a major factor in the development and acceleration of kidney disease in diabetic patients (Saud *et al*., 2009). Report from the Third National Health and Nutrition Examination Survey (NHANES III, 2002) indicates that there are 8,000,000 individuals in the U.S. with significantly decreased kidney function (seventh report of joint National Committee, 2003). It is evident that most patients with chronic kidney disease do not progress to end stage renal disease, but likely succumb to cardiovascular disease, which is also the leading cause of mortality of end stage renal disease patients on maintenance dialysis (Sowers J. *et al*., 2001).

The first stage of chronic kidney disease is called incipient nephropathy defined by microalbuminuria without overt proteinuria (30–300 mg/24 hr or 20–200 microgram/min, or a protein:creatinine ratio >30 mg/mmol, or an albumin: creatinine ratio of 3.4–30 mg/mmol). This phase is often accompanied by glomerular hyperfiltration (GFR $\geqslant$ 90 ml/min), with a serum creatinine lower than expected for age and weight (Rulan et al., 2007; NHFA, 2008).

As the disease progresses, more albumin leaks into the urine and this stage may be called macroalbuminuria or proteinuria. As the amount of albumin in the urine increases, the kidneys' filtering function mildly begins to drop (GFR of 60–89 ml/min) as the body retains various wastes (Diabetes Care, 2005). A greater degree and progressive increase in proteinuria correlates strongly with the progression of renal failure. As kidney damage develops, blood pressure also rises and because normal GFR falls with age therefore many normal older people may fall into this category (Diabetes Care, 2005).

The third stage is associated with a moderately decreased GFR (30–59 ml/min), stage four with severely decreased GFR (15–29 ml/min), and stage five or kidney failure with a very severe GFR less than 15 (Krolewski *et al.*, 1996). Overall, kidney damage rarely occurs in the first 10 years of diabetes, and usually 15 to 25 years will pass before kidney failure occurs. For people who live with diabetes for more than 25 years without any signs of kidney failure, the risk of ever developing it decreases (Diabetes Care, 2005).A vast array of signs and symptoms listed below may manifest when kidney disease has progressed i.e. protein in the urine, high blood pressure, ankle and leg swelling, leg cramps, frequent urination often at night, high levels of serum and urine urea nitrogen (BUN) and serum creatinine, less need for insulin or antidiabetic medications, morning sickness, nausea, and vomiting, weakness, paleness, anemia and itching (Saud *et al*., 2009). Chronic renal failure is a sustained and irreversible reduction in the glomerular filtration rate (GFR), accompanied by a rise in serum creatinine of >200 micromol/L or calculated GFR <60 ml/min on two occasions at least a month apart in the absence of acute illness while end-stage renal disease is usually reached when less than 10% of normal renal function remains and regular dialysis or renal transplantation is required to maintain life (WHO, 2009).

CHAPTER THRE

3.0. MATERIALS AND METHODS

3.1 Study setting

The study was conducted at Moi Teaching and Referral Hospital (MTRH) diabetic clinic. The hospital is located along Nandi Road in Eldoret Town, Uasin Gishu county in Rift Valley. MTRH serves patients from the Western Region which includes Rift Valley, Nyanza and Western Provinces. The clinic day is every Tuesday and Thursday and on average attends to an average of 150 patients on a typical clinic day.

The clinic staff comprises nurses, medical officers and specialist physicians. The nurse first does the blood sugar tests on patients, and then the medical officer attends to them and may recommend specimen collection for tests before drug prescription. The costs of the tests and drugs are met by the patient. If complications are suspected the medical officer then refers them to a specialist physician for further evaluation. The normal routine tests include the determination of the blood sugar, creatinine, and the urine protein.

3.2. Study design

The study design was cross-sectional.

3.3. Study population

The study population was adult diabetic patients attending MTRH diabetic clinic.

3.3.1. Sample size

The sample size for a population-based cross-sectional study was determined using three factors: (i) the estimated prevalence of the variable of interest – diabetics attending referral hospital, (ii) the desired level of confidence and (iii) the acceptable margin of error. The sample size required can be calculated according to the following formula and parameters (Fisher *et al.*, 1991

Formula:

$$n = \frac{t^2 \times p(1-p)}{m^2} \qquad (1.1)$$

Parameters:

n = required sample size

t = tabulated variate at at 95% (standard value of 1.96)

p = estimated prevalence of diabetic patients suffering from hypertension

m = margin of error at 5% (standard value of 0.05)

Example

In the Moi Teaching and Referral, it has been estimated that roughly 30% (0.3) of the Diabetic patients suffer from hypertension. This figure has been taken from national statistics on Hypertension in rural areas. Use of the standard values listed above provides the following calculation.

Calculation:

$$n = \frac{1.96^2 \times 0.3(1-0.3)}{0.05^2}$$
$$n = \frac{3.8416 \times .21}{.0025}$$
$$n = 322.72 = 323 \quad (1.2)$$

3.5. Inclusion criteria

i. Diagnosed diabetes mellitus.

ii. Age ≥18 and ≤70 years.

iii. For normotensives; (BP ≤ 120/80 mmHg).

iv. Pre-hypertensives; (BP 120/80 - 139/89 mmHg).

v. Hypertensives: (BP ≥140/90 mmHg).

3.6. Exclusion criteria

i. Severe illness requiring hospitalization as judged by the medical officer.

ii. History of renal dialysis.

iii. History of smoking

iv. Pregnancy at time of study

v. HIV infection

3.7. Study Methods

3.7.1. Recruitment of study participants and specimen collection

Convenience sampling was used where all patients visiting the clinic were initially included in the study between 8.00 am and 2.00pm., twice a week (Tuesday and Thursday). All individuals aged 18 to 70 were then screened for the exclusion and inclusion criteria by the physician. . Study was carried out from December 2011 to April 2012. The socio demographic data were collected from the hospital records and entered into data collection forms by the investigator. 5mls of venous blood was aseptically collected in a sterile EDTA vacutainer tubes (Beckon Dickson) by a trained medical phlebotomist from MTRH and 10mls of urine was also collected using sterilized evacuated tubes and then mixed with tartaric acid. Urine samples were collected between 8.00am and 1.00pm and labeled with a code number that has a link with the consent form but not an individual participant and then stored at room Temperature (20-25^{0}C) until all the tests are done and completed within 24 hours of collection.

3.7.2. Laboratory Analysis.

The blood and urine samples were then transported to the Biochemistry laboratory at room temperature (20-25^{0}C) and analysed for creatinine, urea, protein and glucose levels by the investigator. Laboratory procedures was performed according to protocol as outlined in appendix IV; serum and urine were analysed using Diacetylmonoxime, Jaffe's, Sulphosalicyclic acid and Urinometer methods to obtain creatinine, urea, protein and glucose levels.

3.7.3. Data management and Analysis

Data was collected by the investigator and entered into data collecting forms through laboratory results printouts and hospital records. Counterchecking was done by the investigator to ensure that data was entered correctly to avoid errors. The data collected was further subjected to inferential analysis tests. The numerical data was expressed as arithmetic mean, standard deviation and frequency distribution. The kidney function was calculated by correlating the creatinine, urea, protein and glucose levels between the hypertensive and non hypertensive diabetics. Chi-square test goodness of fit with confidence limit of 95% (P= 0.05) was used to draw conclusion concerning the relationships and differences found in research results.

3.8. Ethical considerations

The study had been reviewed and approved by Institutional Review Ethics Committee of Moi Teaching and Referral Hospital and Moi University before commencement.

Participants meeting the study criteria were explained the purpose of study in a language they could understand. They were informed about the procedure of the study, risks, benefits, confidentiality, voluntariness and rights to refuse or withdraw without any penalty, those who refuse were not discriminated in the usual care. Contacts of the investigator were provided in the informed consent form (appendix III). A written informed consent was obtained from those who agreed to participate. There was no direct benefit in participating in the research but the results obtained will be useful in designing better guidelines in the predisposing risk factors and the management of hypertension, diabetes and chronic kidney disease.

The study posed no major risks or harm because serum and urine is usually collected during routine tests. The respondents were assured that all tests to be carried will be kept confidential. No name was used in both the samples and in the results to be published.

CHAPTER FOUR

4.0. RESULTS

Table 1: Demographic Characteristics of the Study Groups

Characteristics		**N = 162** **Diabetic & hypertensive**	**N = 162** **Diabetic & Non Hypertensive**	**P Values**
Age (x)		59.94 (SD=4.91)	60.75 (SD=4.62)	0.932
Sex	Male	81 (25%)	81 (25%)	
	Female	81(25%)	81 (25%)	
Weight(x)		65.2 (SD=4.62)	66.7 (SD=4.75)	0.935

Three hundred twenty four participants (162 males, 162 females) of mean age 60.35±4.75 years (diabetic hypertensive subjects were 59.9±4.91 years and diabetic non hypertensive were 60.75 ±4.62 years) and mean weight 65.95 Kgs (diabetic hypertensive subjects being 65.2±4.62 Kgs and diabetic non-hypertensive being 66.7 ±4.75 Kgs.) were recruited.162 (81 males, 81 females) were controls while 162 (81 males, 81 females) were the study group. Generally, there was no significant difference (P>0.05) in demographic characteristics between the two study groups (Table 1).

Table 2: Serum Creatinine, Urea, Glucose, and proteins in the study groups

Parameters	**Diabetic & hypertensive**	**Diabetic & Non Hypertensive**	**P Values**
Creatinine (mg/dl)	2.3	1.5	0.053
Urea 15-40mg/dl	57.8	17.3	0.042*
Glucose (mmol/L)	9.2	8.6	0.171
Proteins	296.45	234.56	0.062

*Significant P-values

There was a difference in serum creatinine, urea, glucose, and proteins in the study groups as shown in Table 2. Significant difference (F=0.042, P=0.05) in serum urea was observed between the two study groups with hypertensive diabetics having a mean of 57.8 mg/dl while the non-hypertensive group had a mean of 17.3 mg/dl. However, no significant difference was observed with regard to creatinine (2.3 or 1.5), glucose (9.2 or 8.6), and protein (296.45 or 234.56) mg/dl levels in serum between the two study groups. This meant that either category of patients were physiologically similar in waste generation.

Table 3: Urine Creatinine, Urea, Glucose, and proteins in the study groups

Parameters	**Diabetic & hypertensive**	**Diabetic & Non Hypertensive**	**P Values**
Creatinine (mg/dl)	2.43	1.55	0.05*
Urea 7-20 (mg/dl)	19.24	15.3	0.045*
Glucose 2.5-5 (mmol/L)	5.7	5.5	0.171
Proteins (mg/24 hours)	3700	2500	0.052

*Significant P-values

The levels of urine creatinine, urea, glucose, and proteins in the study groups showed an identical trend as depicted in Table 3. Significant differences (P =0.05) in urine creatinine and urea (P=0.045) levels was observed between the two study groups. The Hypertensive study group had higher creatine level of 2.43 mg/dl compared to that of non-hypertensive control group which was 1.55 mg/dl. On the other hand, 19.24 mg/dl and 15.3 mg/dl urea levels were observed in the Hypertensive and non-hypertensive study groups respectively. However, no significant difference was observed with regard to creatinine (1.5or 2.43), glucose (5.7 or 5.5), and protein (3700 or 2500) levels in serum between the two study groups.

Table 4: Serum Creatinine, Urea, Glucose, and proteins in the study group in relation to stage of hypertension

Parameters	**Diabetic & hypertensive**			**P Values**
	Normal blood pressure (≤ 120/80)	**Pre-hypertensive (120/80 – 139/89 mmHg)**	**High blood pressure (≥140/90 mmHg)**	
Creatinine (mg/dl)	1.46	1.67	2.52	0.089
Urea 15-40mg/dl	21	37	54.7	0.091
Glucose (mmol/L)	17.3	24.5	28.3	0.123
Proteins (mg/dl)	235.45	244.6	294.7	0.058

*Significant P-values

The serum creatinine, urea, glucose, and proteins in the study group data in relation to three degrees of hypertension was as shown in Table 4. Although not significant, a general increase trend of all the serum study parameters from the non-hypertensive's (systole/diastole of at or below 120/80) which were 1.46,21, 17.3, 235.45 respectively to pre-hypertensive (systole 120 to 139 & a diastolic of 80 to 89) which were 1.67, 37, 24.5, 244.6 respectively with highest levels being observed in the high blood pressure subjects (systole 140 or higher & diastole of 90 or higher) of 2.52, 54.7, 28.3, 294.7 respectively.

Table 5: Urine Creatinine, Urea, Glucose, and proteins sub analysis in the study group in relation to stage of hypertension

Parameters	**Diabetic & hypertensive**			**P Values**
	Normal blood pressure (≤120/80 mmHg)	**Pre-hypertensive (120/80 – 139/89 mmHg)**	**High blood pressure (≥140/90 mmHg)**	
Creatinine (mg/dl)	1.37	2.23	2.29	0.127
Urea (mg/dl)	17.6	19.7	23.5	0.167
Glucose (mmol/L)	5.5	5.4	5.3	0.173
Proteins (mg/dl)	3590	3630	3820	0.085

*Significant P-values

An analogous analysis of urine Creatinine, Urea, Glucose, and proteins in the study group in relation to the three degrees of hypertension are given in Table 5. Similarly, as serum tests in various categories of blood pressure, urine tests for the study variables were not significant. The study also observed a general increase trend of the study parameters except glucose from the non-hypertensive's (systole/diastole of at or below 120/80) to pre-hypertensive (systole 120 to 139 & a diastolic of 80 to 89) with highest levels observed in the high blood pressure subjects (systole 140 or higher & diastole of 90 or higher). This meant that either category of patients were physiologically similar in waste generation.

CHAPTER FIVE

5.0. DISCUSSION

Both serum and urine analysis tried to explain how blood pressure would influence renal disease damage and alter laboratory indicators of kidney integrity. The data supported the view that hypertension defined by high blood pressure may contribute to decrease of the integrity of the kidneys. The demographic characteristics in the two study groups especially weight may not have biased the study results since all participants were approximately 60 Kg in weight. There was no difference in the weight of the study participants; hence, weight may not have contributed to the creatinine, urea, glucose and protein levels observed. Overweight condition is associated with renal hyperfiltration and hyper perfusion, irrespective of the presence of hypertension, and that obesity magnifies the effect of hypertension on albuminuria (Saiki *et al*., 2005), thus raising the possibility of an increased susceptibility of obese hypertensive patients to the development of renal damage. The prevalence of hypertension has also been shown to increase linearly as in patients without renal disease in patients with higher body weight (Pavkov *et al*., 2006). The patients used were not obese per se.

The indication of renal dysfunction in patients with diabetes and hypertension was based on serum creatine, urea, proteins and glucose and/or the detection of urinary excretion of these parameters. Significant difference in serum urea was observed between the two study groups with hypertensive diabetics. Significant difference in urine creatinine and urea levels was observed between the two study groups. The kidney's are the key to understanding why a person's creatinine level would be high (Wolf and Ziyadeh, 1999; Adler et al., 2003). Often, elevated levels of creatinine point directly to the body's

filtration system and how that system is functioning (Hilgers and Veelken, 2005). Of the greatest concern is a decrease in kidney function. Damage to the body's filtration can lead to a higher than normal or elevated creatinine level, and all this has a strong connection to kidney function. In support of the present study, other studies have shown that hypertension, and diabetes can cause damage to kidneys filtration ability (N.H.F.A., 2008). Therefore, the fact that one has an elevated creatinine level could be an indication of something far more important about the body, including treatments (Ritz and Orth, 1999b; Wolf and Ziyadeh, 1999).

Although not significant, the present study has also shown higher urine protein levels of 3700 mg/24 hours in hypertensive diabetics compared to the non hypertensive cases of 2500 mg/ 24 hours. In fact, a similar study observed significant association (P=0.041) between hypertension and proteins in urine, especially in type I diabetes patients *(*Tapp *et al*., 2004.). Tight control of blood pressure is essential for the reduction of urine proteins as well as further micro- and macro-vascular diabetic complications (Wolf and Ziyadeh, 1999). Kidney function has been shown to depend on the intact function of the intricate glomerular micro-vasculature (Ritz and Orth, 1999b; Wolf and Ziyadeh, 1999). While an elevated serum creatinine concentration points to a reduced rate of glomerular filtration, an increased rate of protein excretion points to a derangement in the glomerular filtration barrier (Adler et al., 2003; Tapp *et al*., 2004.). The presence of proteinuria in patients with treated essential hypertension has been shown to vary between 4% and 16% in different series of treated hypertensive patients (Tapp *et al.*, 2004. ; Pavkov *et al*., 2006). Proteins in urine are a well-known predictor of poor renal outcomes in patients with type

2 diabetes and in essential hypertension (Wolf and Ziyadeh, 1999; Hilgers and Veelken, 2005).

The present study has shown that the levels of glucose do not to vary significantly with regard to hypertension and non-hypertension in diabetic patients. Normally excreted by the kidney, the concentration of glucose and advanced glycosylated products is inversely proportionate to the glomerular filtration rate (Odetti *et al*., 2013). However, though kidney damage is irreversible, early intervention in the management of hyperglycemia has been shown to halt the progression of glomerular damage and stabilize kidney function (National Diabetes Information Clearinghouse, 2007). In support, Hall also observed that tight glucose control normalizes and stabilizes kidney (Hall, 2006). These previous study do not vary much from the present study in that glucose level don't show varied difference in the integrity of kidneys of the two study categories.

High blood pressure is one of the leading causes of kidney failure, also called end-stage renal disease (Ritz and Orth, 1999b) since it forces molecules through the kidneys glomerular. This is indicated by the high levels of urine creatinine, urea, and proteins in diabetic hypertensive patient. Although not significant, the study observed a general increase trend of all the serum and urine study parameters from the non-hypertensive's to pre-hypertensive with highest levels being observed in the high blood pressure subjects. This observation could be because hypertension is present in approximately 80 to 85 percent of patients with chronic kidney disease (American Diabetes Association, 2007). Elevations in blood pressure within the normal range also occur in patients with chronic glomerular disease and may be clinically significant which is similar to the present study (Rossing, 1995; NHFA, 2008). In addition, patients with end-stage renal disease are more

likely to have an increase in these variables and isolated systolic hypertension (Diabetes Spectrum, 2006). Why this occurs is incompletely understood but increased aortic stiffness appears to play an important role (Wolf and Ziyadeh, 1999). Patients with chronic kidney disease may not demonstrate the normal nocturnal decline in blood pressure, a possible risk factor for hypertensive complications.

CHAPTER 6

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1.Conclusion

i. There was significant difference in serum urea (F=0.042, P=0.05) between the two study groups.

ii. There was significant difference in urine creatinine (P= 0.05) and urea (P= 0.045) between the two study groups.

iii. There was a general increasing trend though not significant of serum creatinine, urea, glucose, and proteins from non hypertensives (1.46,21, 17.3, 235.45 respectively) to pre-hypertensives (1.67, 37, 24.5, 244.6 respectively) with highest levels being observed in the high blood pressure subjects (2.52, 54.7, 28.3, 294.7 respectively). Urine tests for the study variables were not significant. The results also indicated a general increase trend of the study parameters except glucose from the non-hypertensive's (systole/diastole of at or below 120/80) to pre-hypertensive (systole 120 to 139 & a diastolic of 80 to 89) respectively; with highest levels observed in the high blood pressure subjects (systole $\geq$ 140 & diastole of $\geq$ 90).

iv. There was significant difference in the kidney functionality as indicated by the significant differences in serum urea and urine creatinine and urea. There was no

significant differences in serum creatinine, glucose and proteins and in urine Glucose, and proteins

6.2. Recommendations

The study recommends the following:-

1. Early screening for hypertension both before and after the onset of diabetes, early treatment, aggressive glycemic control, patient education and close monitoring can largely avert kidney disease or kidney failure due to diabetes and hypertension.

2. There is need for prosthetic studies to establish whether the control or duration of hypertension worsens kidney disease.

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APPENDICES

Appendix I: Consent to Participate in Research Studies

EFFECT OF HYPERTENSION ON RENAL FUNCTIONS OF DIABETICS ATTENDING MTRH IN KENYA

INVESTIGATOR: Lucy Jepkurui Bargerei, Tel: 0720792142

University of Eldoret

School of Science

Department of Biological Sciences

P.O. Box 1125-30100, Eldoret.

PURPOSE AND BACKGROUND: I am an MPhil student of University of Eldoret. Iam carrying out a study to determine the effects of hypertension on kidney function among diabetics. 117 of diabetics with hypertension with 117 of diabetics without hypertension are expected to participate. Iam approaching you because my study targets the patients visiting the diabetic clinic.

I kindly request you to allow us to take some blood and urine samples if you accept. The blood will be aseptically collected in a sterile EDTA vacutainer tubes (Beckson Dickson) by an expert and apart from this there is no effect. You will also be given a container to provide urine for analysis. The blood and urine samples will be used to establish the effects of hypertension on renal functions of diabetic patients.

BENEFITS: There will be no direct benefit in participating in this research but the results will be useful in designing better guidelines in the management of chronic kidney disease.

RISKS: The study will not pose any risk besides the slight pain associated with drawing venous blood.

CONFIDENTIALITY: I am assuring you that at least all tests to be carried will be kept confidential. There will also be no name appearing in both the samples and in the results to be published.

QUESTIONS: Incase of further questions, comments/complaints relating to research, contact me (the investigator) through: Address: Lucy J. Bargerei, P.o Box 6615, Eldoret.

VOLUNTARY PARTICIPATION: My participation in this study is entirely voluntary and i am free to accept or refuse to take part without any penalty or discrimination.

PROCEDURE: I agree to participate. 5ml of venous blood will be drawn asceptically from me in EDTA vacutainer tubes and 10ml of urine using sterilized evacuated tubes.

CONSENT: I have read/heard the nature of the study and i voluntarily agree to participate in the study

You will be required to sign this form as evidence of acceptance.

1. Patient Signature……..Date……..

2. Investigator Signature …….Date……..

Appendix II: Data to be collected

Data	Method
Medical history	Pertinent Medical History in the Initial Evaluation of Hypertension: Symptoms suggesting secondary hypertension History of high blood pressure, including duration and levels Results and side effects of previous antihypertensive therapy Use of oral contraceptives, steroids, NSAIDs, nasal decongestants, appetite suppressants, tricyclic/tetracyclic antidepressants, MAO inhibitors, cocaine and other illicit drugs, alcohol, and/or herbal supplements History of tobacco use, diabetes, hyperlipidemia History of weight gain, exercise, sodium and fat intake History or symptoms of stroke, transient ischemic attack, angina, previous myocardial infarction, coronary revascularization procedure, heart failure, claudication, renal disease Family history of coronary artery disease, stroke, renal disease and hypertension Psychosocial and environmental factors that may influence blood pressure

	Snoring, daytime somnolence
Blood Pressure	Screening and identification of elevated blood pressure > 140/90* BP Classification SBP mmHg DBP mmHg Normal < 120 and < 80 Prehypertension 120-139 or 80-89 Stage 1 hypertension 140-159 or 90-99 Stage 2 hypertension > 160 or > 100 Blood pressure goal Change treatment: Add a second drug from another class Substitute an agent from another class Increase the dose of the initial drug

Urine characteristics	Creatinine (mg/dl) (estimate GFR*) Urea (mg/dl) Protein Glucose
Secondary hypertension.	Chronic kidney disease/obstructive uropathy Thyroid and parathyroid disease Drugs (prescription, over-the-counter, herbal supplements, illicit drugs) Excessive alcohol use Obstructive sleep apnea Primary aldosteronism Renal artery stenosis Pheochromocytoma Cushing's syndrome Aortic coarctation Obesity

Appendix III: Data Entry form

Table 6: Data entry form

Age	Gender	Weight (kgs)	Blood Pressure	Serum and rine test results			
				Creatinine (gm/dl)	Urea (gm/dl)	Proteins (gm/dl)	Glucose

Appendix IV: Laboratory procedure

Urea – Diacetyl monoxime method

Principle

Urea reacts directly with diacetyl monoxime under strong acidic conditions to give a yellow condensation product. The reaction was intensified by the presence of ferric ions and thiosemicarbazide. The intense red colour formed is measured at 540nm/ yellow green filter.

Reagents

All chemicals were be Analar grade.

Stock acid reagent

1.0g of ferric chloride hexahydrate was dissolved in 30 ml of distilled water. 20 ml orthophosphoric acid was then added and mixed. This was stored in a brown bottle at room temperature (25-35^{0}C).Stable for 6 months.

Mixed acid reagent

Slowly 100 ml of Conc. H2S04 was added to 400 ml distilled water taken in a 1-litre flat-bottom conical flask kept in an icecold waterbath and mixed well. Then 0.3ml of stock acid reagent was added, mixed and stored in a brown bottle at room temperature (25-35^{0}C). Stable for 6 months.

Stock colour reagent – A

2g diacetyl monoxime was dissolved in distilled water and the volume made up to 100 ml in a volumetric flask then stored in a brown bottle at room temperature (25-35^{0}C). Stable for 6 months.

Stock colour reagent - B

0.5 g thiosemicarbazide was dissolved in distilled water and made up to 100 ml in a volumetric flask then stored in a brown bottle at room temperature (25-35^{0}C). Stable for 6 months.

Stock urea standard

1.0g of analytical-grade urea was dissolved in 100ml of benzoic acid (1g/dl). 100ml of volumetric flask was used for preparing this and stored at room temperature (25-35^{0}C). Stable for 6 months.

Working standard 50mg/dl

5.0ml of stock urea standard was diluted to 100 ml with benzoic acid then stored at room temperature (25-35^{0}C). Stable for 6 months.

Procedure

The protocol of the procedure is described below.

The following was pippeted into appropriately labelled 13 x 100 mm tubes and mixed well (Dilution of Standards (S1-S3), Test & QC)

	S1	S2	S3	Test	QC
Distilled Water (ml)	1.9	1.8	1.7	1.9	1.9
50 mg/dl Urea (ml)	0.1	0.2	0.3	-	-
Test sample /QC (ml)	-	-	-	0.1	0.1

Colour Development

The colour reagent was prepared fresh at the time of analysis by mixing distilled water, mixed acid reagent and mixed colour reagent in the ratio 1:1:1.

The following was pippeted into another set of appropriately labelled 18 x 150 mm tubes and mixed well.

	Blank	S1	S2	S3	Test	QC
Colour reagent (ml)	3.1	3.0	3.0	3.0	3.0	3.0
Respective diluted standard (ml)	-	0.1	0.1	0.1	-	-
Diluted test /QC (ml)	-	-	-	-	0.1	0.1

The tubes were kept in a boiling waterbath for 15 minutes, then removed from the waterbath and cooled for 5 minutes. The spectrophometer/filter photometer was set to

zero with blank at 540nm/yellow green filter and the absorbance of the other tubes measured.

Calculation and calibration graph

Concentration of standards:

S1=50mg/dl

S2=100mg/dl

S3 = 150 mg/dl

The absorbance values of standards against their respective concentrations were plotted in a graph. The measurable range with this graph was from 10 to 150 mg/dl. A calibration graph was constructed whenever a new set of reagents was prepared. The absorbance values of test/QC werel then plotted on the calibration graph and the concentrations read off. Once linearity was proved, it was enough if S3 were set up every time those patients' samples were analysed and the results calculated using the formula:

Absorbance of the test

Urea in test sample = ……x 50 mg/dl

Absorbance of standard (WHO, 2009).

3.9.2. Creatinine – Jaffe's method

Principle

Creatinine present in serum or urine directly reacts with alkaline picrate resulting in the formation of a red colour, the intensity of which is measured at 505nm/green filter.

Protein interference is eliminated using sodium lauryl sulphate. A second absorbance reading after acidifying with 30% acetic acid corrects for non-specific chromogens in the samples.

Reagents

All chemicals will be Analar grade

(a) Reagent A

Into 400ml of distilled water taken in a 500 ml beaker, 4.4g of NaOH was added and mixed to dissolve, then 9.5g trisodium phosphate [Na3PO412H2O] was added and mixed. Then 9.5g of sodium tetraborate [Na2B4O710H2O] was added. After dissolving the pH was checked and if above 10, it's was adjusted if necessary by the dropwise addition of 1M NaOH. This was transferred into a 500 ml volume flask and made up to 500ml with distilled water then mixed well. Stable for 3 months at 2-8^{0}C.

(b) Reagent B

20g sodium lauryl sulfate was dissolved in a final volume of 500ml distilled water. Stable for 6 months at room temperature (25-35^{0}C).

(c) Reagent C

4.6g of anhydrous picric acid was required. Therefore approximately 7.0g but not less than 6.0g moist picric acid was weighed and added to 500ml of distilled water taken in a volumetric flask, mixed and left overnight at 370 C. Then filtered and stored in brown glass bottle at room temperature (25-35^{0}C). Stable for 1 year.

(d) Working reagent

At the time of analysis freshly mixed equal volumes of the above three reagents was used and after any leftover working reagent discarded.

(e) Stock creatinine standard 100mg/dl

100 mg of pure creatinine in 0.1 M HCl was dissolved and made up to 100 ml with 0.1 M HCl in a volumetric flask. Stable for 6 months at 2-8^0 C.

(f) Working creatinine standard

2, 4, 6 and 8 ml of stock creatinine standard was dissolved each to 100 ml with 0.1 M HCl to get creatinine concentrations of 2, 4, 6 and 8 mg/dl, respectively. Stable for 6 months at 2-8^{0}C.

(g) 30% (V/V) Acetic acid

30ml of glacial acetic acid was dissolved to 100ml with distilled water. Stable for 3 months at room temperature (25-35^{0}C).

Procedure

The protocol of the procedure is described below.

The following was Pipetted into appropriately labeled 18 x 150 mm tubes and mixed well. (Standards: S2 =2mg/dl, S4=4mg/dl, S6=6mg/dl & S8=8mg/dl)

	Blank	**S1**	**S2**	**S3**	**Test**	**QC**
Working reagent (ml)	3.0	3.0	3.0	3.0	3.0	3.0
Distilled Water (ml)	0.2	-	-	-	-	-
Standard (ml)	-	0.2	0.2	0.2	-	-
Test sample /QC (ml)	-	-	-	-	0.2	0.2

Then left at room temperature (25-35^{0}C) for 30 minutes. The spectrophotometer/ filter photometer was set to zero with blank at 505 nm/green filter and the absorbance of the other tubes measured. After measuring the absorbance the solutions were poured back into the respective tubes. Then added 0.2 ml of 30% acetic acid to the test and QC tubes, mixed well and left at room temperature (25-350C) for 5 minutes. Again the spectrophotometer/filter photometer was set to zero with blank at 505nm/green filter and the absorbance of test and QC measured.

Calculation and calibration graph

The second absorbance values of test and QC was subtracted from the first set of values. Then a calibration graph by plotting the absorbance values of standards against their respective concentrations drawn. The measurable range with this graph was from 0.2 to 8.0 mg/dl. The corrected absorbance of test and QC was plotted and the values of creatinine read off.

Once linearity was proved, it was enough if a single standard such as S6 was taken each time when patients' samples were analysed and the results were calculated using the following

Formula

$$\text{Serum Creatinine} = \frac{\text{Test absorbance}}{\text{Standard absorbance}} \times 6 \text{ mg/dl}$$

Protein- Sulphosalicylic acid method

Principle

Urine proteins were precipitated by sulphosalicylic acid, which gives a white precipitate, and the degree of the precipitate was proportional to the protein level.

Reagents

7.5 g of sulphosalicylic acid was weighed and dissolved in about 200ml of distilled water and then made up to 250 ml with distilled water then stored at 25 - 35^0C. Stable for 6 months.

Procedure

To 2ml of urine taken in a 13 x 100mm glass tube, 2 ml of 3g% SSA was added and mixed gently then left for 5 minutes at room temperature. The degree of the precipitate was compared with 4ml of SSA taken in a similar test tube.

Results

Colour change	**Result**
No cloudiness	Negative
Faint cloudiness (observed only if the tube was held against a black background).	Trace
Definite nongranular cloud without flocculation	1+
Heavy and granular cloud without flocculation	2+
Dense cloud with marked flocculation	3+
Thick curdy flocculation & coagulation	4+

Interpretation quality control

The sulphosalicylic acid method did not detect protein in normal urine, but was sensitive enough to detect protein present down to 20mg%. As a quality control measure, a 22g/dl albumin solution was diluted appropriately with 0.9 g/dl sodium chloride to get standards containing 20, 50, 200, 500 and 2500 mg/dl proteins. These standards were stable for one

month when stored at 2-8^{0}C. When they were subjected to the same procedure as urine, the results could be interpreted as follows:

Concentration of proteins	**Reported as**
20 mg/dl	Trace
50 mg/dl	1+
200 mg/dl	2+
500 mg/dl	3+
2500 mg/dl	4+

Appendix V: Formal 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
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Tel: 33471/2/3

7th November, 2011

Reference: IREC/2011/93
Approval Number: 000748

Lucy J. Bargerei,
Moi University,
School of Science,
P.O. Box 4606-30100,
ELDORET - KENYA.

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
APPROVED
07 NOV 2011
P.O. Box 4606 ELDORET

Dear Ms. Bargerei,

FORMAL APPROVAL

The Institutional Research and Ethics Committee has reviewed your research proposal titled:-

"Effect of Hypertension on Renal Functions of Diabetics Attending a Referral Hospital in Kenya."

Your proposal has been granted a Formal Approval Number: **FAN: IREC 000748** on 6th November, 2011. You are therefore permitted to begin your investigations.

Note that this approval is for 1 year; it will thus expire on 5th November, 2012. If it is necessary to continue with this research beyond the expiry date, a request for continuation should be made in writing to IREC Secretariat two months prior to the expiry date.

You are required to submit progress report(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.

Yours Sincerely,

DR. W. ARUASA
AG. CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

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