Estimation of Kidney Injury Molecule-1 and Microalbuminuria levels in Hypertensive patients at the University Teaching Hospital, Lusaka

By

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A Dissertation Submitted to the University of Zambia, in Partial Fulfilment of the Requirements for the Master of Science Degree in Pathology (Chemical Pathology)

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Declaration

I Mildred Zulu hereby declare that with the exception of the assistance acknowledged, the work being submitted for the master of Science Degree in pathology (Chemical pathology) at the University of Zambia, Lusaka is entirely my own work. I also declare that it has not been presented either wholly or in part, for any other degree or diploma and is not being currently submitted for any other degree at this or other Universities.

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Certificate of Approval

Dissertation Title: Estimation of Kidney Injury-Molecule-1 and Microalbuminuria levels in Hypertensive patients at the University Teaching Hospital, Lusaka, Zambia

This dissertation of **Mildred Zulu** has been approved in partial fulfillment of the requirements for the Master of Science degree in pathology (Chemical pathology) at the University of Zambia.

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Abstract

Context— Acute kidney injury (AKI) and chronic kidney disease (CKD) represent one of the important health problems with high morbidity and mortality in Zambia. Recognition of AKI allows prompt injury specific intervention that may avert permanent renal damage and progression of CKD to End Stage renal Disease (ESRD) can also be delayed with early diagnosis and intervention. Hypertension is a major public health problem and the most important cause of renal failure and dialysis all over the world. Kidney injury molecule-1 (KIM-1) and Microalbuminuria (MAU) have been shown to be important biomarkers of kidney injury. There is less data on the levels of KIM-1 and Microalbuminuria in hypertensive patients at the University Teaching Hospital (UTH).

Aims— the aim of the study was to determine the levels of kidney injury molecule-1 and microalbuminuria in hypertensive participants at the UTH.

Methods and Results— an analytical cross section study was undertaken at the UTH. 40 hypertensive participants and 40 non hypertensive participants were enrolled to the study; the renal function tests (serum creatinine, urea, electrolytes) were determined using Olympus AU480 chemistry analyser. Urinary KIM-1 and MAU concentrations were determined using ELISA kits. Our results showed that there was no difference in KIM-1 levels between hypertensive participants (2.817 ± 1.359ng/mL) and non hypertensive participants (3.286 ± 1.143ng/mL) with p =0.122. However, the hypertensive participants had higher MAU levels (130.809 ± 84.744 µg/mL) than non hypertensive participants (15.983 ± 20.442µg/mL), with $p < 0.001$. KIM-1 showed a positive correlation with MAU in hypertensive participants with statistical significance ($r = 0.326$, $p = 0.045$). However KIM-1 showed a weak negative correlation with creatinine ($r = -0.279$, $p = 0.09$), whereas MAU was positively correlated with creatinine in hypertensive participants with statistical significance ($r = 0.556$, $p = 0.001$).

Conclusion— there was no difference in KIM-1 levels between hypertensive participants and non hypertensive participants. Hypertensive participants had higher MAU concentration than non hypertensive participants. The positive correlation between MAU and Creatinine could indicate that they can both be used to detect kidney injury in hypertensive individuals. In Zambian setting creatinine and Microalbuminuria are still the biomarkers of choice for the diagnosis of kidney injury. However, Urinary KIM-1 could provide diagnostic and prognostic advantages by providing information on the reversibility of kidney injury following treatment.

Dedication

With great love I dedicate this dissertation to my mother Ireen Jere , my dad Bernard Zulu, my husband Kabuno Biemba and my daughter Tabo, for their unconditional love, spiritual and emotional support during my whole time of my study and for their desire to see me succeed. May I always make you proud.

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This research study could not have been a success without direct and indirect contribution from many people. It is therefore not possible to exhaust the list of their names. In this regard, for those who may not be specified by name, please accept my sincere apologies.

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LIST OF ABBREVIATIONS

AKI	Acute Kidney Injury
AKIN	Acute Kidney Injury Network
ARF	Acute Renal Failure
B.P	Blood Pressure
BUN	Blood Urea Nitrogen
CI	Confidence Interval
Conc.	Concentration
CKD	Chronic Kidney Disease
DF	Degree of Freedom
Dr	Doctor
DRGS	Director of Research and Graduate Studies
ECM	Extracellular Matrics
ESRD	End Stage Renal Disease
ELISA	Enzyme Linked Immunosorbent Assay
GBD	Global Burden of Diseases
GFR	Glomerular Filtration Rate
HIV	Human Immuno-defficient Virus
HRP	Horseradish Peroxidase
IBM	International Business Machines
KDa	Kilodalton
KI	Kidney Injury
KIM-1	Kidney Injury Molecule-1
KS	Kaposi Sarcoma
MAU	Microalbuminuria
Ml	millilitres
mmHg	Millimetres of mercury
NAG	N-acetyl –β-glucosamidase
ng	Nanogram
nm	Nanometre
O.D	Optical Density
RAAS	Renin Angiotensin Aldosterone System
RPM	Rounds per minute
RRT	Renal replacement Therapy

SCr	Serum Creatinine
S.D	Standard Deviation
SPSS	Statistical Package for Social Sciences
TGF-β	Transforming Growth Factor β
TIM-1	T-cell immunoglobulin and mucin- containing molecule-1
TMB	3, 3', 5, 5' Tetramethylbenzidine
UAE	Urinary Albumin Excretion
Vs	Versus
UNZABREC	University of Zambia Biomedical Research Ethics Committee
USA	United States of America
UTH	University Teaching Hospital
WHO	World Health Organisation

1.0 INTRODUCTION

1.1. BACKGROUND

Kidney injury is a condition in which the kidneys fail to function adequately. In physiology, it is described as a decrease in the glomerular filtration rate (Liangos *et al.,* 2007). There are two types of renal injury; acute kidney injury (AKI) and chronic kidney disease (CKD) which are indicative of the rate at which damage occurs, rather than the mechanisms by which it occurs. AKI is defined as the rapid or abrupt deteriorating in the function of the kidney which clinically manifest as reversible acute increase in nitrogenous waste products measured by blood urea nitrogen and serum creatinine levels with or without reduced urine output over the course of hours to less than three months in a patient whose renal function was previously normal (Schrier *et al*., 2004; Vaidya *et al*., 2008).

Recently, a consortium of nephrologists and intensivists, the Acute Kidney Injury Network (AKIN) recommended the change of the name from acute renal failure (ARF) to "acute kidney injury" (AKI) (Van Timmeren *et al.,* 2006). This term includes the entire spectrum of ARF and recognizes that minor changes in kidney function (reflected by a change in serum creatinine [SCr] of 0.3 mg/dL) can have worse patient outcome, whereas the term "failure" is reserved for those patients whose renal functional impairment is so severe that replacement therapy is indicated, or at least considered (Bonventre, 2010).

Acute kidney injury occurs in approximately 5% -7%of all hospitalized patients and up to 30% of admissions to intensive care units and historically it has been associated with a high risk of morbidity and mortality. It represents a significant and devastating problem in clinical medicine (Gordfarb and Scott, 2001).

The persons at risk of developing AKI are older persons, those with pre-existing chronic kidney disease, hypertensive patients , sepsis, diabetes, heart disease, liver disease and also those taking nephrotoxic drugs such as; Tenofovir, Non-steroid anti-inflammatory drugs and aminoglycosides to mention a few. The major medical complication of AKI is progression to chronic kidney disease and end stage renal disease (Burtis *et al*., 2012).

Chronic renal failure, also called chronic kidney disease, is kidney damage which has been present for three months or more as defined by structural or functional abnormalities of the kidney with or without decreased glomerular filtration rate, manifesting either pathological abnormalities or markers of kidney damage, including abnormalities in the composition of blood or urine, or abnormalities in imaging tests (Levey *et al*., 2007). CKD is a worldwide public health problem leading to a steadily increasing number of patients with end stage renal

disease. It is 100 times more prevalent than end-stage renal disease, and its incidence is increasing at an even faster rate (Qiu-Li and Dietrich, 2008). The groups at high-risk of CKD include patients who have a family history of the disease and patients who have diabetes, hypertension, recurrent urinary tract infections, urinary obstruction, or a systemic illness that affects the kidneys (London, 2003).

Hypertension is an increasing important medical and public health issue in both developed as well as developing world, affecting one in every four people. People with hypertension, are at increased risk for developing kidney disease which could lead to eventual kidney failure and death (Robert *et al.*, 2011). This is hard to predict in patients who do not regularly check the status of their kidney function. Therefore, some may undergo serious kidney damage without knowledge or diagnosis.

The identification of reliable biomarkers for kidney injury would be useful to facilitate early intervention, evaluate the effectiveness of therapeutic interventions, and guide pharmaceutical development by providing an indicator for nephrotoxicity (Han *et al*., 2002; Ibrahim *et al*., 2013)

The standard methods of assessing renal function have kept the measurement of blood urea nitrogen and creatinine, biomarkers that are insensitive and nonspecific, especially in the setting of AKI (Ibrahim *et al*., 2013; Mori and Nakao, 2007). Furthermore, it is important to recognize that changes in serum creatinine and blood urea nitrogen concentrations primarily reflect functional changes in filtration capacity and are not true 'injury markers' (Bonventre, 2009). In highlight of the inadequacies of these routine biomarkers, there is a critical need for more reliable biomarkers of AKI for its timely diagnosis, prediction of severity and outcome, and for the monitoring of proximal tubule injury in AKI and also progression in CKD (Ogrizovi, 2010).

Microalbuminuria (MAU) refers to an abnormally increased excretion rate of albumin in urine that is below the threshold of detection by the conventional urinary dipstick (30–300 µg/ml) (Toto, 2004; Ferguson *et al*., 2008). In hypertension MAU is associated with increased mortality. It has been described as an early sign of kidney damage, end stage renal disease (ESRD) and risk of developing cardiovascular disease in renal patients (Poudel *et al.,* 2012).

Kidney injury molecule-1 (KIM-1) is a Type I transmembrane glycoprotein with a cleavable ectodomain (90 kDa) which is localized in the apical membrane of dilated tubules in acute and chronic injury. It is minimally expressed in healthy kidneys but is rapidly up-regulated

and expressed on the apical surface of renal epithelial cells of the proximal tubule in response to injury (Fontanilla *et al.,* 2011).

The urinary levels of kidney injury molecule-1 and microalbuminuria have not been studied in Zambia particularly the University Teaching Hospital among hypertensive patients. This study therefore aimed at investigating the levels of Kidney injury molecule-1 and microalbuminuria in hypertensive patients

1.2. STATEMENT OF THE PROBLEM

According to the World Health Report 2002 and Global Burden of Disease project, diseases of the kidney and urinary tract contribute to the global burden of diseases, with approximately 850,000 deaths every year and 15,010,167 disability-adjusted life years. They are the 12^{th} cause of death and the 17^{th} cause of disability, respectively (Schieppati and Remuzzi, 2005). In Zambia, Kidney disease is the number 27^{th} cause of death among the major 50 causes of death (WHO, 2014).

The cost of renal replacement therapies that include dialysis and transplantation are very high; in developing countries such as Zambia, hence prevention of KI progressing to end stage renal disease is often the only cost effective way to decrease its severe impact on morbidity and mortality. This can only be achieved when you have knowledge on its early detection. Early recognition would allow prompt injury specific intervention that may avert permanent renal damage and delay progression of CKD and ESRD. The early detection can only be done through the use of reliable biomarkers. At the UTH, laboratory diagnosis of KI is mostly done by the measurements of Serum Creatinine, blood urea nitrogen and electrolytes; however these biomarkers are non specific especially in the setting of AKI. Kidney injury molecule-1 (KIM-1) a type 1 transmembrane glycoprotein has been shown to be an important biomarker of kidney injury (Nickolas *et al*., 2008).

Among hypertensive patients the prevalence of microalbuminuria is about 25% and is higher than that observed in diabetic patients (20%) (Pontremoli *et al*., 2005; Polónia *et al*., 2007). In hypertensive patients the relationship between microalbuminuria and impaired renal function is still uncertain, although several studies have shown microalbuminuria to be an important early marker of kidney disease in patients with hypertension.

The urinary levels of kidney injury molecule-1 and microalbuminuria have not been studied in Zambia particularly in hypertensive patients at the UTH.

1.3. JUSTIFICATION OF THE STUDY

This study would contribute to the understanding of the association between KI and KIM-1 as well as microalbuminuria levels among hypertensive Zambians. The results of this study would also help enhance the efforts of policy makers and clinicians to control and manage kidney disease as well as to facilitate early intervention and initiation of therapy so as to reduce the morbidity, mortality and costs associated with KI and improve the quality of life of patients.

In hypertensive patients microalbuminuria has long been considered a marker of early kidney injury and increased cardiovascular risk. For this reason, detection of microalbuminuria could be useful in selecting specific therapeutic strategies for reducing and preventing cardiovascular and renal events in hypertensive patients.Therefore the study would add knowledge to the field of medicine in Zambia.

To assess the levels of KIM-1 and microalbuminuria in hypertensive patients, an analytical cross section study was undertaken. To the best of my knowledge, this was the first study of its kind in Zambia.

1.4.0 LITERATURE REVIEW

1.4.1 Prevalence of Kidney Disease

Prevalence of kidney injury particularly CKD and ESRD is on rise worldwide (Yirsaw, 2012), it remains under-diagnosed and under-treated since in its early stages the disease is often asymptomatic, making individuals with the disease and also their health care providers unaware of its silent yet threatening presence (Naicker, 2003). The prevalence of renal failure or kidney injury ranges from 1.1% to 20% worldwide with United States of America (USA) having 3.5 -12%, Europe, Israel and Argentina 3.5-4.7%, India 27% and Iran 20% (Naicker, 2013).

In the outpatient renal screening studies that have been done in Sub-Saharan Africa describe varying prevalence of renal dysfunction ranging from 6% to 50%. Screening outpatient studies in South Africa reported 6% and 5.5% renal dysfunction, 33.5% in Zambia and 48.5% in Uganda (Fabian and Naicker, 2009). In Sub-Sahara Africa renal dysfunction affects mainly those aged 20-50 years primarily due to hypertension and glomerular disease (Akinsola *et al.,* 2004).

In a study done by Banda *et al*. (2010) at the UTH of Zambia showed a prevalence of renal dysfunction of 42% among hospitalised Human Immuno-defficient virus (HIV) positive infected patients compared to 27% among non HIV infected patients.

1.4.2 Pathogenesis of Acute Kidney Injury

AKI is a life threatening disease that is one of the major causes of morbidity and mortality in hospitalized patients. It represents an acute decline in renal function that leads to structural changes and can progress to CKD and end stage renal failure if not detected early (Michael *et al.,* 2009).

The pathogenesis of AKI is complex and to some extent, varies based on the particular cause; however, many convergent processes lead to tissue and organ dysfunctional. Causes associated with toxins also have a final common pathway contributing to local or generalized ischemia. AKI is a state often characterized by enhanced intrarenal vasoconstriction; it is also associated with enhanced renal-nerve activity and increased tissue levels of vasoconstrictive agents, such as angiotensin and endothelin (Bonventre, 2010). Gordfarb and Scott (2001) explained that a decreased responsiveness in the resistance vessels to vasodilators such as acetylcholine, bradykinin and nitric oxide as well as lower production levels of some vasodilators can enhance the impact of these vasoconstrictive agents.

These effects on the resistance vessels are complemented by endothelial damage, enhanced leukocyte-endothelial adhesion (particularly in the post capillary venules), and activation of the coagulation pathways; together these processes result in small vessel occlusion and further activation of the leukocytes causing increases in inflammation and providing a positive feedback network (Burtis *et al.,* 2012). The inflammation produces increased levels of mediators expanding the interactions between leukocyte and endothelial cells and activating the coagulation pathway. The resultant effects on oxygen and nutrient delivery to the epithelial cells result in damage to those cells furthermore; damaged tubular cells also generate proinflammatory mediators. Repair involves the replacement of lost cells in the tubule by mechanisms that are not completely understood (Tesch, 2010).

1.4.3 Pathogenesis of Chronic Kidney Disease

Chronic kidney disease (CKD) is the progressive loss of renal function caused by a heterogeneous group of diseases but involving a common final pathophysiological process. CKD results from irreversible loss of glomeruli by glomerulosclerosis, a process consisting mainly of glomerular scarring (Patel *et al*., 2002).

Swedko *et al.* (2003) revealed a gender-different prevalence of CKD; with higher prevalence of CKD in females than males. Females have less muscle mass as compared to males and the muscle mass is a major determinant of serum creatinine concentration.

According to Foley (2006), the major causes of CKD are commonly related to systemic vascular diseases, like diabetes, hypertension or atherosclerosis. Other diseases that contribute to CKD include toxin exposure, immune complex deposition and autoimmune diseases.

Regardless of the type of the primary cause, the pathological sequence of events involved in CKD is almost the same. The initiating factor causes decrease in the number of nephrons leading to structural and functional changes in the remaining surviving nephrons to compensate for the nephrons loss (Louis, 2009). Adaptive mechanisms initially occur to increase the blood flow to the non sclerosed glomeruli and hence maintain a normal GFR. This is called hyper filtration and it is mediated by vasoactive mediators, renin-angiotensin aldosterone system (RAAS), cytokines, transforming growth factor β (TGF-β) as well as by other growth factors (Schieppati and Remuzzi, 2005).

This adaptive mechanism leads to increase in the pressure in the remaining glomeruli and cause their accelerated sclerosis leading to further loss of the nephrons number. Further

adaptation by hyper filtration overwhelms the remaining normal nephrons that will be at further risk of sclerosis. Hence, chronic kidney disease progresses in a self-perpetuating way (Robert *et al*., 2011).

Levey *et al*. (2007) further explained that as the number of nephrons decreases more and more, the glomerular filtration rate (GFR) further decreases and renal shrinkage occurs. When the GFR dramatically decreases, symptoms of uraemia start and the patient would be having ESRD (a condition requiring dialysis or kidney transplantation to maintain patients' long-term survival)

1.4.4 Hypertension and kidney disease

Hypertension is a major public health problem and the most important cause of renal injury and dialysis all over the world. It is present in more than 80% of patients with CKD and contributes to progression of kidney disease toward ESRD as well as to cardiovascular events such as heart attack and stroke. Hypertension is a leading cause of CKD in Sub Sahara Africa ranging from 25% in Senegal, 29.8% in Nigeria, 45.6%% in South Africa and 48.7% in Ghana (Kwangu *et al.,* 2014). In SA hypertension affects about 25% of the adult population and is the cause of ESRD in 21% of patients on renal replacement therapy (RRT). In Zambia the prevalence of hypertension was found to be 34.8% (38.0% males and 33.3% female) (Goma *et al.,* 2011).

The kidneys have a central role in the control of sodium homeostasis through the important mechanism of regulation of blood pressure (Rodriguez-Iturbe *et al*., 2005). Hypertension produces clinical proteinuria and a significant reduction in renal function in 5 – 15% of patients. Moreover, 25% of patients with end stage renal disease have hypertension as the primary diagnosis. Huge prevalence of hypertension in the general population still remains the second leading cause of end-stage renal disease (ESRD), with the risk being substantially higher in blacks. The mechanism leading to renal damage involves Tubulo-interstitial injury which seems to be one of the main histological determinants of hypertension-related kidney damage (Rodriguez-Iturbe *et al*., 2005). It was initially thought that interstitial and tubular damage was secondary to glomerular and vascular injury and occurred in the final stages of hypertensive nephropathy (Bakris *et al*., 2010). However, Barkris *et al*. (2010) demonstrated that this type of kidney damage could be present in hypertensive patients before any changes in glomerular vessels.

1.4.5 Microalbuminuria and kidney disease in hypertension

Albumin is the most abundant protein in the circulation and during normal kidney function very little intact albumin is excreted by the kidney (<30 mg/ day in humans). However, following renal injury, glomerular filtration of albumin is increased and the reabsorption and degradation of albumin by tubules are decreased, resulting in increased levels of intact albumin in the urine (i.e. albuminuria) (Cohuet and Struijker-Boudier, 2006).Patient albuminuria is usually classified by ranges of severity, which are: microalbuminuria (30–300 mg/day), macroalbuminuria (300 mg–3 g/day) and nephritic range albuminuria (>3 g/day). Albuminuria is commonly used as an early marker of renal injury because it often precedes a decline in renal function (Tesch, 2010).

According to Cohuet and Struijker-Boudier (2006), increased urinary albumin excretion has been associated with several unfavourable metabolic and non metabolic risk factors and subclinical hypertensive organ damage. Microalbuminuria has a higher prevalence in hypertensive subjects than in the general population and there is convincing evidence of independent relationships between its presence and diastolic blood pressure levels, pulse pressure, and isolated systolic hypertension (Verdecchia and Reboldi, 2004). Experimental and clinical studies recognise two major causes for the increased urinary albumin excretion (UAE) in hypertension; hemodynamic changes leading to elevation in intraglomerular pressure due to the direct transmission of increased systemic pressure to the glomeruli and generalised angiopathy, related to endothelial dysfunction, characterised by renal and systemic transvascular albumin leakage (Verdecchia and Reboldi, 2004).

1.4.6 Kidney injury molecule-1 in Kidney injury

KIM-1 (also known as TIM-1—T-cell immunoglobulin and mucin-containing molecule-1) is a type 1 transmembrane structural glycoprotein which contains, in its extracellular portion, a novel six-cysteine immunoglobulin-like domain, two N-glycosylation sites and a T/SP rich domain characteristic of mucin-like O-glycosylated proteins. It is located in the renal proximal tubule epithelial cells.Tubular epithelial express KIM-1 at the apical membrane once injured by difference forms of renal injury (Hilde *et al*., 2012). Metalloproteinase slice KIM-1 into a soluble part and a short membrane-bound fragment while the tubular epithelial cells produce various proinflammatory cytokines and chemokines. These cytokines and chemokines draw inflammatory cells to the renal interstitium and initiate interactions with interstitial fibroblasts, activation and proliferation of fibroblasts and myofibroblasts leading

to excessive synthesis of extracellular matrix (ECM) and eventually to fibrosis. With ongoing injury, the tubular epithelial cells can undergo programmed cell death (apoptosis). Apoptotic bodies express phosphatedylserine on their surfaces (Kramer *et al.,* 2009). KIM-1-expressing tubular epithelial cells can bind to surface-specific epitopes on the apoptotic bodies, specifically to phosphatedylserine, and can phagocytose dying cells and other debris from the tubular lumen (Ichimura *et al*., 2008). In addition to the facilitation of clearance of the apoptotic debris from the tubular lumen, KIM-1 play an important role in limiting the autoimmune response to injury since it is known in many systems that phagocytosis of apoptotic bodies is one mechanism for limiting the proinflammatory response (Malyszko *et al.,* 2010).There is still disagreement about the function of KIM-1; is it actively regulating the inflammation, or is it involved in the process of repair and/or damage or is just a result of ischemia, proteinuria or renal fibrosis? (Mirjan *et al*., 2007).

The soluble form of human KIM-1 can be detected in the urine of patients with Acute Tubular Necrosis and also in those with CKD and may serve as a useful biomarker for renal proximal tubule injury facilitating the early diagnosis of the disease (Han *et al*., 2005). Furthermore, high urinary KIM-1 expression was evaluated prospectively in a cohort of 201 hospitalized patients with AKI and was also associated with adverse clinical outcome (death and need for dialysis) in patients with AKI (Mai and Prasad, 2008). Although KIM-1 gene or protein expression is undetectable in the normal kidney, following injury KIM-1 mRNA is rapidly synthesized and protein is generated and localized at very high levels in the apical membrane of proximal tubule in the region where the tubule is most affected (Ali *et al.,* 2007). In human ischemic and toxic AKI, it is found in all three segments of the proximal tubule. The KIM-1 expression is absent in the glomerulus, peritubular interstitial cells, or inner medullary cells (Bonventre, 2009).

In spite of the reported protective functions of KIM-1 in AKI, there are a number of evidences for its roles in the chronic injury in CKD. Kidney injury molecule-1 was expressed in differentiated tubular epithelium in CKD similar to in AKI, which also suggested a role of KIM-1 in tubular fibrosis in CKD (Bonventre, 2009). KIM-1 expression is also seen in several malignancies including renal cell carcinoma and clear-cell ovarian carcinoma. The ectodomain of KIM-1 undergoes regulated cleavage, appearing in the urine where it is stable and readily detected by commercially available enzyme-linked immunosorbent assay. It is believed to function as a cell adhesion molecule in the process of regenerating and reconstructing damaged proximal tubules. Presence of KIM-1 in the urine is highly specific for kidney injury. No other organ has been shown to express KIM-1 to a degree that would

influence kidney excretion. It has been shown to be much more sensitive than creatinine as a marker for AKI (Ibrahim *et al.,* 2013).

In current clinical practice, kidney injury is typically diagnosed by measuring serum creatinine and blood urea nitrogen. Both urea and creatinine are products of protein metabolism which are cleared almost entirely by the kidneys (Bailly *et al.,* 2002). Blood urea nitrogen (BUN) is measured in serum; however, its levels are affected by non-renal influences such as protein intake, dehydration, liver function, gastrointestinal bleeding and steroid use (Ruggenenti *et al.,* 2001).

Creatinine levels are also affected by non-renal influences such as muscle mass, age, gender, muscle metabolism, protein intake and liver function (Bjomsson, 1979; Vaidya *et al.,* 2006). These biomarkers are insensitive to early kidney damage; increase in creatinine levels is seen when 60-75% of nephrons are non-functional while increase in urea is seen when approximately 75% of the nephrons become malfunctional, and therefore, these markers are not reliable for the diagnosis of kidney damage (Doi *et al.,* 2009).

Therefore, there is need for the use of biomarkers that could be used to detect renal injury, and this may affect timely diagnosis and, possibly, reduce the progression of kidney disease to end stage renal failure. These biomarkers (creatinine, urea and electrolyte are measured in blood, therefore in this regard, the quantitative determination of KIM-1 and Microalbuminuria in urine using a specific ELISA kit might be considered a non-invasive and objective method to easily and rapidly identify kidney injury or disease in human subjects.

1.5 Research Question

Does kidney injury result in increased urinary KIM-1 and Microalbuminuria levels in hypertensive participants?

2.0. OBJECTIVES

2.1 General Objective

To determine the levels of kidney injury molecule-1 and microalbuminuria in hypertensive patients at the University Teaching Hospital.

2.2 Specific Objectives

1. To determine the mean difference in urinary kidney injury molecule-1 concentration between hypertensive participants and non hypertensive participants
2. To determine the mean difference in Microalbuminuria concentration between hypertensive individuals and non hypertensive individual
3. To assess for any possible correlation between KIM-1, Microalbuminuria and Creatinine as biomarkers of kidney injury in hypertensive participant

3.0. METHODOLOGY

3.1. STUDY DESIGN

An analytical cross section study was conducted involving hypertensive patients attending renal unit (clinic 5) of the University Teaching Hospital (UTH), Lusaka, Zambia.

3.2 STUDY SITE

The study was conducted at the hypertensive renal unit (Clinic 5) of the University Teaching Hospital. The UTH is the highest referral hospital in Zambia located in Lusaka. It receives referral patients from districts and provincial hospitals from all over Zambia and from the health centres within Lusaka. The renal unit (clinic 5) at UTH is a specialist clinic consisting of renal patients that have been referred to UTH for further management; it runs a weekly outpatient clinic for renal patients on Fridays.

3.3 TARGET AND STUDY POPULATIONS

The target population was confirmed hypertensive patients with confirmed kidney injury attending out-patients renal unit (clinic 5) at UTH who were 18 years and above.

The study population was confirmed hypertensive participant with confirmed kidney injury who met the inclusion criteria.

3.4 SAMPLE SIZE

A calculated sample size was **126 (63 hypertensive patients with kidney injury or renal dysfunction and 63 clinically normal participants)** using the formula for determination of sample size for comparative research studies between two groups as given below;

$$N = \frac{4\sigma^2(z_{crit} + z_{pwr})^2}{D^2},$$

where N is the total sample size (the sum of the sizes of both groups), σ is 6 ng/ml the assumed SD of KIM-1 of each group (assumed to be equal for both groups), the zcrit value is 1.960 as given in tables for the desired significance criterion of 0.05 or 95% confidence interval (CI), the zpwr value is 0.842 as given in Standard Normal Deviate (zpwr) tables corresponding to 80% statistical power, and D is the minimum expected difference between the two means which has been estimated at 3ng/ml. Both zcrit and zpwr are cut off points along the x axis of a standard normal probability distribution that demarcate probabilities matching the specified significance criterion and statistical power, respectively.

Due to inadequate reagents a total sample size of 80 was collected, 40 cases and 40 controls

3.5 SAMPLING METHOD

In this study, convenience sampling method was used. Recruitment was done on Friday during normal working hours when the clinic was in operation.

3.6 CASE DEFINITION

Hypertension was defined as follows

Blood Pressure (BP) ≥ 140/90 mm Hg on at least three different occasions or by the presence of antihypertensive treatment.

Normotensive individuals were considered to be:

(a) Those with no previous diagnosis of or treatment for hypertension and with Bp < 140/90

Kidney injury or renal dysfunction

Kidney injury was defined according to the clinical diagnosis; the study included participants already diagnosed clinically as having acute kidney injury or chronic kidney disease. This data was obtained from the participants file.

Serum creatinine, urea, sodium, potassium and chloride were measured to support the clinical diagnosis.

Kidney injury molecule 1 and microalbuminuria levels were then determined and compared in cases and controls.

3.6.1 INCLUSION CRITERIA OF CASES

- Individuals with hypertension and kidney injury or renal dysfunction
- Either gender
- Individuals between the age of 18 and 75
- Those who voluntarily provided written consent after explanation of the study

3.6.2 EXCLUSION CRITERIA OF CASES

- Those who failed to provide written consent to the study
- Hypertensive participants with kidney injury less than 18 years and over the age of 752
- Pregnant women

- Those with other debilitating conditions such as liver disease, diabetes mellitus, cancer, heart failure

NOTE: Non hypertensive participants (controls): Apart from not being hypertensive and not having kidney injury, the inclusion criteria and exclusion criteria for this group were the same as that given for cases.

- The information above was obtained from the participant files and from the interviews administered using a questionnaire

3.7 DATA COLLECTION

3.7.1 Clinical Data

Recruitment of study participants (cases) was done on Friday in the day time when the renal clinic was in progress this was because the renal unit at UTH runs a weekly out-patients clinic on Fridays. Eligible hypertensive patients having renal dysfunction were enrolled to the study. The study was explained and participants who consented by signing the consent form, were recruited and assigned a serial number. Demographic data and medical history was obtained from the participants files. The demographic data included the participants' age, sex, occupation and marital status. The medical history data included the specific date (month or year) in which the participant was diagnosed with hypertension and renal dysfunction and whether the renal dysfunction was acute or chronic, past and current medication, and the presence of medical conditions that may confound the research finding; included here are any major surgeries, diabetes mellitus, cancers and any other debilitating chronic condition.

Participants (study controls) were recruited from healthy individuals who came for medical check-ups at high cost in the UTH during normal clinic hours from Monday to Friday. As the participants were seen by the clinician they were informed and explained to about the study by the clinician who also provided the participants with the study information sheet. When a participant agreed to be part of the study, they were given a consent form to sign and later on assigned a serial number. Thereafter information on the participants' demographic data and medical history was collected and compiled by the researcher. The demographic data for the controls also included the participants' age, sex, occupation and marital status. The cases and the controls were matched according to demographic status (age and sex).

3.7.2 Specimen Collection

Four (4) mLs of blood was collected from the antecubital vein using the vacuum needle from the participants (cases and controls) who had been recruited. The four mLs blood was transferred into Lithium heparin anticoagulated vacutainers (green top). The container was labelled with the serial number that each participant had been assigned at the time of recruitment. The samples were then transported to the clinical chemistry laboratory within UTH immediately for preparation and analysis. The collected blood was used to measure renal function tests (creatinine, urea and electrolytes).

Fresh urine was collected from the consented participants (cases and controls) in sterile urine containers; the urine was then transported to the clinical chemistry laboratory within UTH for preparation and analysis within four hours of collection.

3.7.3 Specimen Preparation and Storage

In the laboratory, each specimen serial number was recorded onto a compilation summary sheet, thereafter a laboratory number or barcode was assigned to each blood specimen. Each blood specimen was logged in the computer system of the UTH clinical chemistry laboratory. After the logging process the blood specimen was centrifuged at 3000 revolutions per minute (3000 rpm) for three minutes in order to separate the plasma (supernatant) from the blood cellular components (sediment).

The centrifuged specimen was then loaded onto the racks in readiness for the measurements of renal function tests which included creatinine, urea and electrolytes (sodium, potassium and chloride).

The urine specimen was transported to the clinical chemistry within four hours after collection to prevent analyte degradation. In the laboratory the urine samples were allowed to sit at room temperature for 30 minutes to sediment, and the supernatant was transferred using Pasteur pipettes to 2mls plastic cryovial containers with sealable screw caps which were then stored in a freezer at -80℃ until the specimens were analysed in a batch.

3.8 Quality Control

To ensure reliable results (accurate, precise and validy), calibration and quality control was performed on all the analytical instruments and analysers to be used for any purpose during specimen analysis according to the UTH quality control guidelines. Quality control included equipment calibration and analytical control runs on every analysis before each test analysis.

3.9.0 Specimen Analysis

3.9.1 Blood pressure measurement

The blood pressure value for both cases and controls were obtained from participants file

3.9.2 Kidney injury molecule 1 Enzyme linked-Immunosorbent assay (ELISA) protocol

Kidney Injury Molecule 1 (KIM-1) concentration was determined using the NeoBioLab®Human HK0032 (United States) ELISA Kit; a quantitative competitive immunoassay for measurement of Human KIM-1 in urine according to the manufacturer's protocol given below. This assay employed an antibody specific for Human KIM-1 coated on a 96-microtitre plate. Standard or experimental samples are co-incubated in wells along with a KIM-1-HRP (Horse radish peroxidase) conjugate. KIM-1 in standards or sample competes with KIM-1-HRP conjugate for binding to the plate bound antibody. Higher levels of KIM-1 from standards or samples leads to decreased KIM-1-HRP conjugate binding and reduced signal. Capture KIM-1-HRP substrate (solutions A and B). Binding of the KIM-1-HRP is visualized by production of colorimetric reaction products that can be quantitatively measured by absorbance at 450nm.

3.9.2.1. Reagent Preparation

All kit components and samples were brought to room temperature before use. The microtiter plate was brought to room temperature before opening. 10 µL of balance solution was dispensed into all experimental sample wells. 10 µL wash solution concentrate (100×) was diluted with 990 mL of distilled water.

3.9.2.2. Assay Procedure

100 µL of sample or standard (KIM-1) (A to F) were added to the appropriate wells in the supplied microtiter plate. There was no addition of blocking steps since the well had been pre-blocked. Then 50 µL of conjugate (Horseradish peroxidase) were added to each microtiter plate and mixed well. The plate was then covered and Incubated for 1 hour at 37°C in a humid chamber. After incubation each well was washed 5 times with 300-400 µL 1X wash solution (Buffered water) per well. After the last wash, the plate was inverted and blotted dry by tapping on absorbent paper to completely remove the liquid at each step. 50 µL of substrate A (Peroxide) was added to each well followed by addition of 50 µL substrate B (3, 3', 5, 5' Tetramethylbenzidine). The plate was here after covered and incubated for 10-15 minutes at room temperature away from direct sunlight covered with a foil. After incubation, 50 µL of stop solution (Acid solution) were added to each well and the contents mixed well. The optical density (O.D.) was immediately read at 450 nm.

3.9.2.3. Data Processing

The O.D. of other non-zero standards were divided by that of the zero standards, and then multiplied by 100 (used as X variables). Then, the base 10 logarithm of other standard concentration was calculated (taken as Y variables). A standard curve was generated from these variables in Microsoft Excel 2011.

Figure 1 : KIM1 Caribration Curve

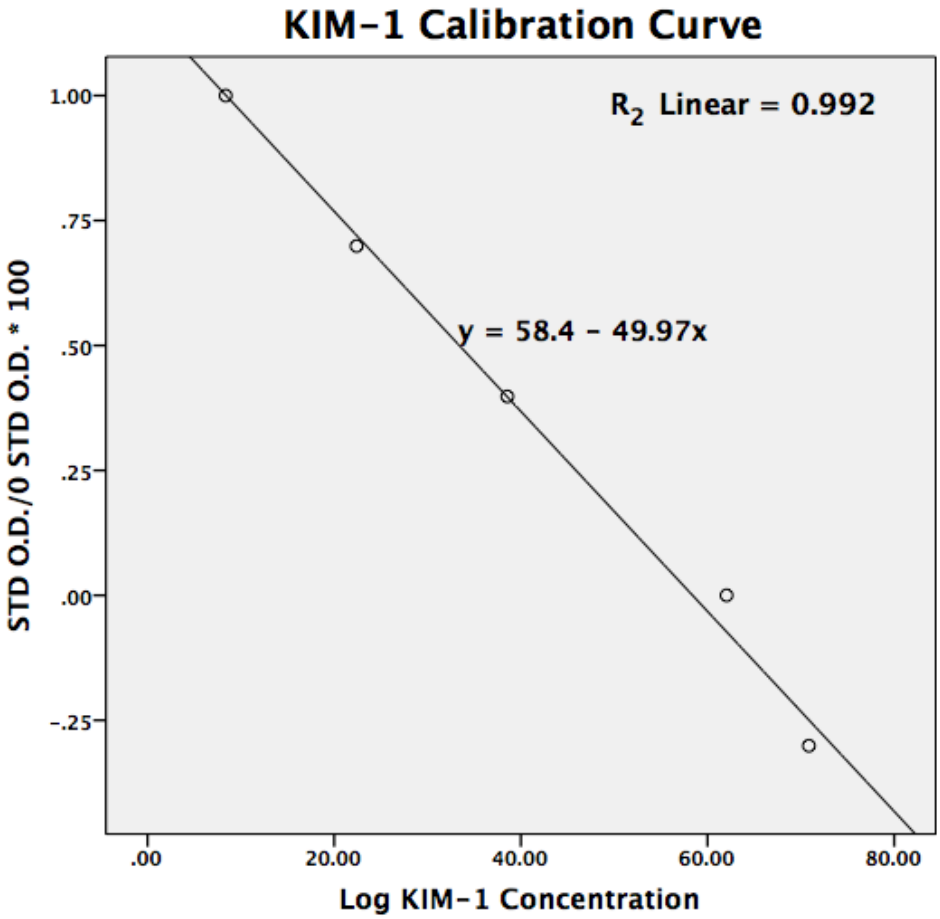

Fig. 1: KIM1 calibration curve plotted from standard absorbances (O.Ds) againist concentrations. The regression equation was used to calculate sample concentration from their respective O.Ds.

To calculate results: the sample O.D. was processed as follows: O.D. of sample divided by that of standard 0, then multiplied by 100, to get Y values using the formulation y = 58.4 – 49.97x. To get the concentration of samples: 10 was powered to Y (10^{Y}).

3.9.2.4. Sensitivity And Specificity

The sensitivity obtained from this ELISA test kit is approximately 0.1 ng/mL. The assay has high sensitivity and excellent specificity for detection of KIM-1. No significant cross-reactivity or interference between KIM-1 and any homologous proteins has been observed. Species cross reactivity has not been specifically determined.

3.9.3 Microalbuminuria ELISA Test Protocol

Microalbuminuria concentration was determined using the Fitzgerald® Industries International (United States) ELISA Kit; a quantitative competitive immunoassay for measurement of Human albumin in urine. This assay employed an antibody specific for Human albumin coated on a 96-well plate. Calibrators, control and urine samples are incubated together with anti-albumin peroxidase conjugate in the wells. If albumin is present, it will compete with coated albumin for the binding of the anti-albumin-conjugate. Washing of the microwells removes unspecific components. Bound enzyme conjugate will hydrolyze the enzyme substrate TMB. The addition of the acid stops the reaction forming a yellow end-product. The intensity of this yellow colour is measured photometrically at 450nm. The amount of colour is inversely proportional to the concentration of albumin present in the original sample.

3.9.3.1. Reagent Preparation

All kit components and samples were brought to room temperature before use. The microtiter plate was brought to room temperature before opening. The wash solution concentrate (50×) was diluted with 990 mL of distilled water up to a final volume of 1000ml prior to use.

3.9.3.2. Assay Procedure

20 µL of Calibrator (A to F), patient sample and controls (negative and positive) were added in to the appropriate wells in the supplied microtiter plate. Then 100 µL of enzyme conjugate were added to each well. The plate was then covered and Incubated for 30 minutes at room temperature. After incubation each well was washed 3 times with 300 µL 1X wash solution (Buffered water) per well. After the last wash, the plate was inverted and blotted dry by tapping on absorbent paper to completely remove the liquid at each step. 100 µL of TMB substrate solution (3, 3', 5, 5' Tetramethylbenzidine) were added to each well. The plate was here after covered and incubated for 15 minutes at room temperature away from direct sunlight. After incubation, 100 µL of stop solution (Acid solution) were added to each well and then incubated for 5 minutes at room temperature. After incubation the optical density (O.D.) was read at 450 nm and the results calculated.

3.9.3.3. Data Processing

The O.D. of other non-zero standards were divided by that of the zero standards, and then multiplied by 100 (used as X variables). Then, the base 10 logarithm of other standard concentration was calculated (taken as Y variables). A standard curve was generated from these variables in Microsoft Excel 2011 for Mac.

FIGURE 2: Microalbuminuria Calibration Curve

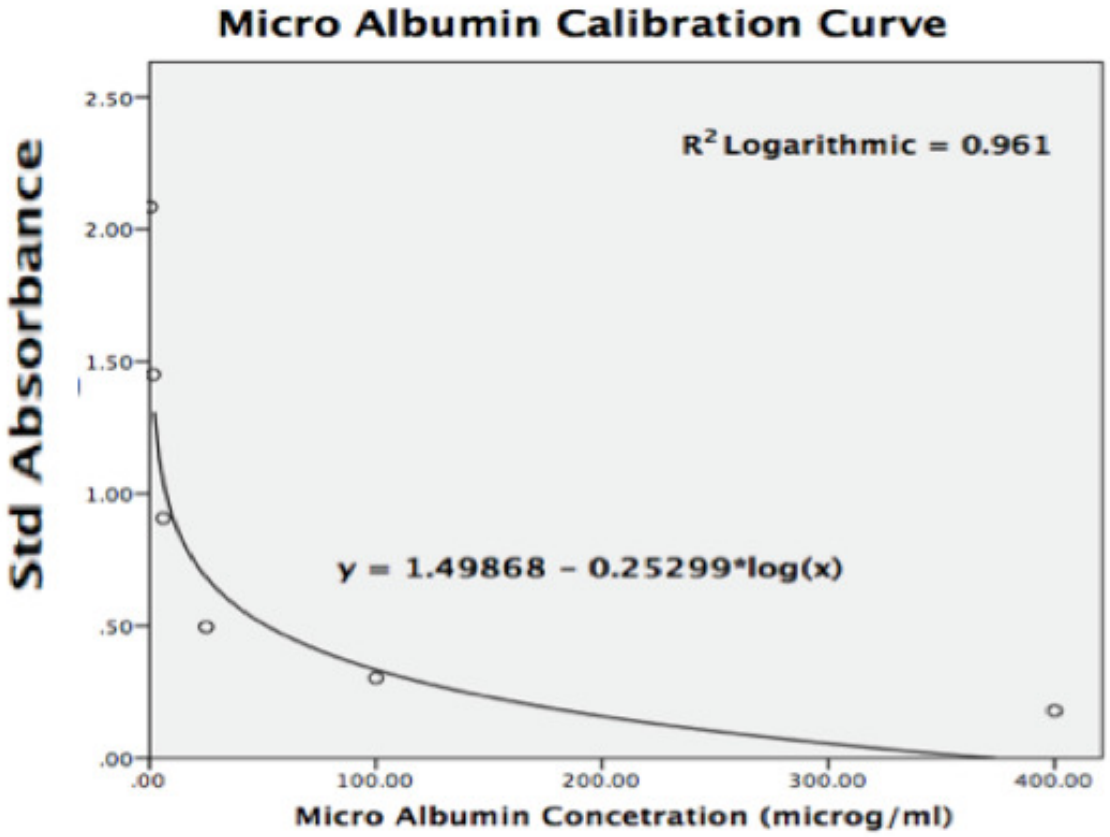

Fig. 2:Microalbuminuria calibration curve plotted from standard absorbances (O.Ds) againist concentrations. The regression equation was used to calculate sample concentration from their respective O.Ds.

To calculate results: the sample O.D. was processed as follows: O.D. of sample divided by that of standard 0, then multiplied by 100, to get Y values using the formulation y = 1.49868 –0.25299 *log(x). To get the concentration of samples: 10 was powered to Y (10^{Y}).

3.9.3.4. Sensitivity And Specificity

Functional sensitivity of the assay is 0.5µg/ml

Both ELISA tests (KIM-1 and Microalbuminuria) were performed in the Kaposi Sarcoma (KS-HHV8) Diagnostic and research Laboratory in the UTH. ELISA plates for urinary KIM-1 and Microalbuminuria were read on the VersaMaxPLUS Rom V1.23 ELISA plate reader.

3.9.4 Creatinine Test Protocol

Serum Creatinine was measured using Bechman Coulter Olympus AU480 chemistry analyser available in the clinical chemistry laboratory in the UTH using the Jaffe method. In alkaline medium Creatinine form a yellow-orange complex with picric acid and the colour intensity directly proportional to the Creatinine concentration in the sample. The Olympus analyser automatically computes the Creatinine concentration of each sample. All test protocols were calibrated and controls ran before samples could be assayed

3.9.5 Urea Test Protocol

Urea was measured using Bechman Coulter Olympus AU480 chemistry analyser available in the clinical chemistry laboratory in the UTH. Urea is hydrolysed by urease to produce carbon dioxide and ammonia. The produced ammonia combines with 2-oxogluterate yielding glutamate and NAD+. The decrease in NADH absorbance per unit time is proportional to the urea concentration. The Olympus analyser automatically computes the urea concentration of each sample. All test protocols were calibrated and controls ran before samples could be assayed

3.9.6 Electrolyte (Sodium, Potassium and Chloride) Test Protocol

Electrolytes were measured using Bechman Coulter Olympus AU480 chemistry analyser available in the clinical chemistry laboratory in the UTH. The Olympus module for sodium, potassium and chloride employs crown ether membrane electrodes for sodium and potassium and a molecular oriented PVC membrane for chloride that is specific for each ion of interest in the sample. An electrical potential is developed according to the Nernst Equation for a specific ion. When compared to an internal reference, this electrical potential is translated into voltage and then into the ion concentration of the sample. The Olympus analyser automatically computes the sodium, potassium and chloride values of each sample. All test protocols were calibrated and controls ran before samples could be assayed.

3.10 ETHICAL CONSIDERATIONS AND PERMISSION

The study was subjected to ethical approval by the University of Zambia Biomedical Research Ethics Committee (UNZABREC) IRB00001131 of IORG0000774 and it was approved (**REF.No. 003-08-14)**. Permission to conduct the study was sought from the UTH Medical Superintendent and the Directorate of Research and Graduate Studies (DRGS) through the Assistant Dean, Postgraduate. Permission to use equipment and facilities in the clinical chemistry laboratory in the UTH was obtained from the Head, Department of Pathology and Microbiology at the University Teaching Hospital as well as from the clinical chemistry laboratory Head of Department, permission to use the ELISA equipment and laboratory facilities in the Kaposi Sarcoma (KS-HHV8) Research and Diagnostic Laboratory was obtained from the Laboratory Head of Department.

Informed consent was obtained from each of the study participants in writing; this was after explaining the study to the participants in private on a one to one basis. The participants were identified by serial number, no names were used. The serial number was given after assigning it using the participant file number. The serial number was the one written on the specimen containers as well as on the cryovial. The file number was obtained so that the results can be returned to the files without difficulties. Venous blood was drawn from consented participants and it was explained that this would cause some discomfort and minimum pain. The blood (4mLs) and urine for study purposes were taken at the same time as any routine bloods or urine requested by the clinician for normal care (e.g. full blood count, urinalysis, urine microscopy, urea, creatinine, e.t.c). This was to minimise venesection. All entry forms (Participants information and result) were kept with strict confidentiality permitted by law and only viewed by approved study personnel. After analysis of the specimens results were returned to the participant files and they were then explained to them by the clinicians. For the non hypertensive participants (controls) they were notified of their results by the researcher or the lab personnel of clinic 7 (High cost Laboratory) in the UTH.

3.11 DATA PROSECING AND STATISTICAL ANALYSIS

Data were expressed as mean ± Standard deviation for normally distributed continuous variables or median (interquartile range) for non-normally distributed variables. Normality was assessed using the Shapiro and Wilk statistic and the normality plots. Skewed variables were log – transformed prior to analysis.

The independent sample student's *t*-test was used to compare mean values of urinary KIM-1 concentration in hypertensive individuals to the normal health controls, mean Microalbuminuria concentration , mean creatinine concentration and other biochemical test (urea, sodium, potassium and chloride) and mean diastolic and systolic blood pressure between the two groups (hypertensive and non-hypertensive group). The data was cleaned and which after showed no violation of normality as assessed by use of the Shapiro and Wilk statistic, and also showed homoscedasticity as assessed by use of the Levene test for equality of variance.

Pearson's correlation was used to assess correlation between KIM-1 and Microalbuminuria, KIM-1 and Creatinine and also between Microalbuminuria and Creatinine in hypertensive participants. When KIM-1 was the dependent variable, the independent variables were Microalbuminuria and Creatinine. When Microalbuminuria was the dependent variable, the independent variables were KIM-1 and Creatinine. The Pearson's correlation data was plotted and presented on scatter graphs

Data were analysed in IBM SPSS Statistics version 22 for Mac and Microsoft Excel 2011. Results were summarised on to tables and graphs as given elsewhere. All statistical tests were performed at 5% significance level or 95% confidence interval and differences were considered significant if 2-tailed $p < 0.05$.

4.0 RESULTS

4.1. KIM1, Microalbuminuria and Creatinine Concentration. Mean Difference in hypertensive versus non hypertensive participants

The study found that there was no difference in KIM-1 concentration between hypertensive participants (2.817 ± 1.359ng/mL) and non hypertensive participants (3.286 ± 1.143ng/mL), $t(69) = -1.565$, $p < 0.122$ (Fig. 3). Microalbuminuria concentration and Creatinine concentration were significantly higher in hypertensive participants (Microalbuminuria; 130.809 ± 84.744 µg/mL and Creatinine ; 597.925 ± 553.304 µmol/L) than in non hypertensive participants (Microalbuminuria; 15.983 ± 20.442µg/mL and Creatinine; 72.361 ± 17.863 µmol/L) with statistical significance, $t(75) = 8.316$, $p = < 0.001$ and $t(74) = 5.930$, $p = < 0.001$ respectively (Fig. 4 and Fig. 5, respectively).

FIGURE 3: KIM-1 Concentration Mean Difference between hypertensives and non-hypertensives participants

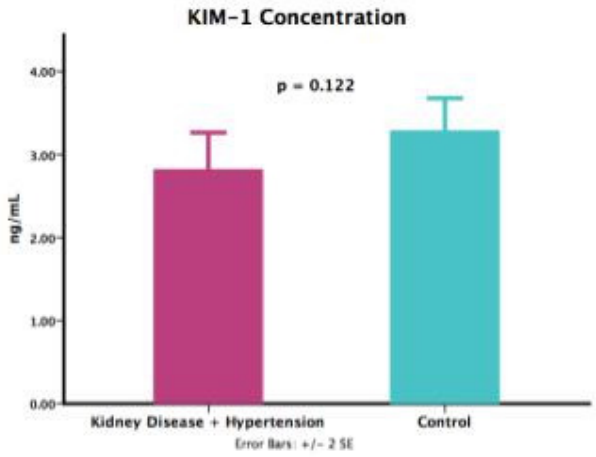

Fig. 3: There was no difference in KIM-1 levels between hypertensive and non-hypertensive participants with ($p = 0.122$)

Figure 4: Microalbuminuria Concentration Mean Difference between hypertensive and non hypertensive participants

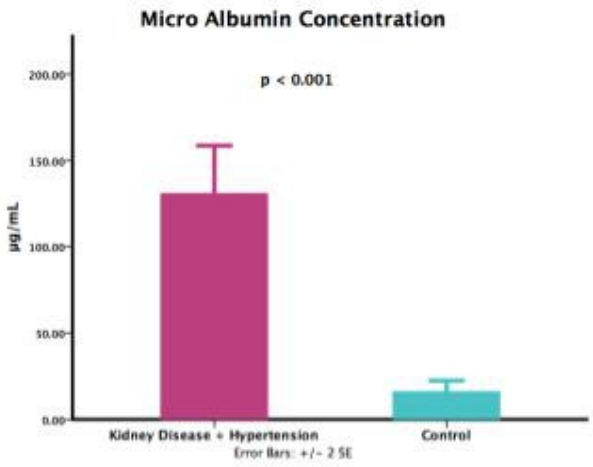

Fig.4: Microalbuminuria levels were significantly higher in hypertensive than in non-hypertensive participants with ($p < 0.001$)

Figure 5: Creatinine Concentration Mean Difference between hypertensive and non-hypertensive participants

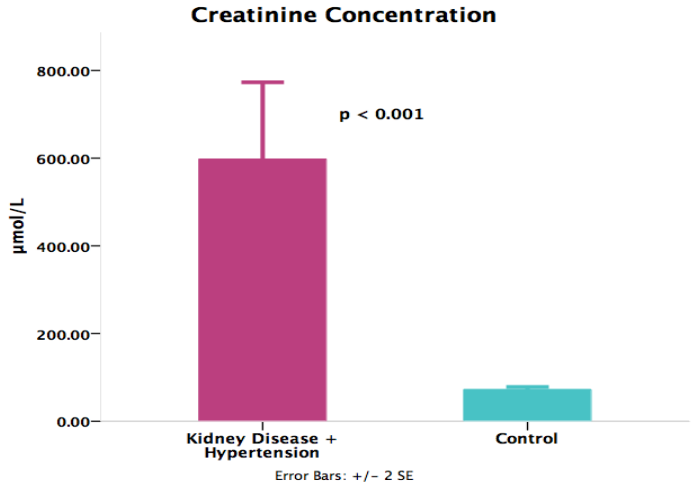

Fig.5: Creatinine levels higher in hypertensive than in non hypertensive individuals with ($p < 0.001$)

4.2 Pearson's Correlation of KIM-1 vs. Microalbuminuria in hypertensive participants

Pearson correlation analysis of KIM-1 vs. Microalbuminuria showed positive correlation in hypertensive participants with statistical significance (r = 0.326, p = 0.045) (Figure 6A).

Figure 6A. Pearson Correlation analysis between urinary KIM-1 and Microalbuminuria in hypertensive participants

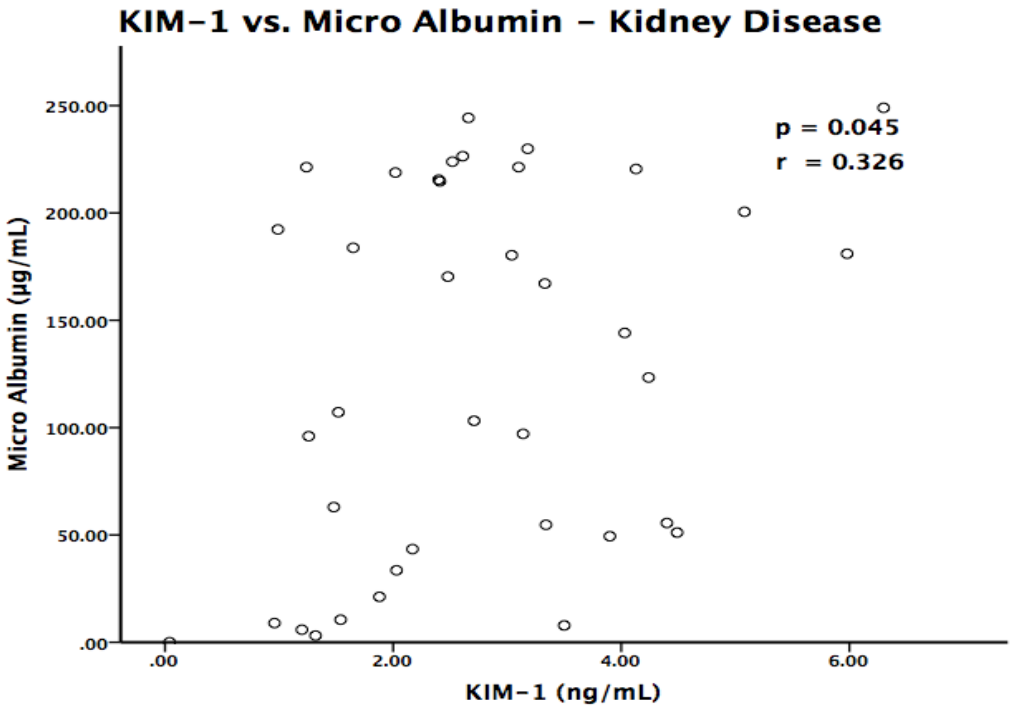

Fig 6A: Positive correlation between KIM-1 and Microalbuminuria in hypertensive participants with p = 0.045

4.3 Pearson Correlation analysis between urinary KIM-1 and Creatinine in hypertensive participants

Pearson correlation analysis of KIM-1 Vs Creatinine showed a weak negative correlation in hypertensive participants without statistical significance (r = -0.279, p = 0.090) (Figure 6B).

Figure 6B. Pearson Correlation analysis between urinary KIM-1 and Creatinine in hypertensive participants

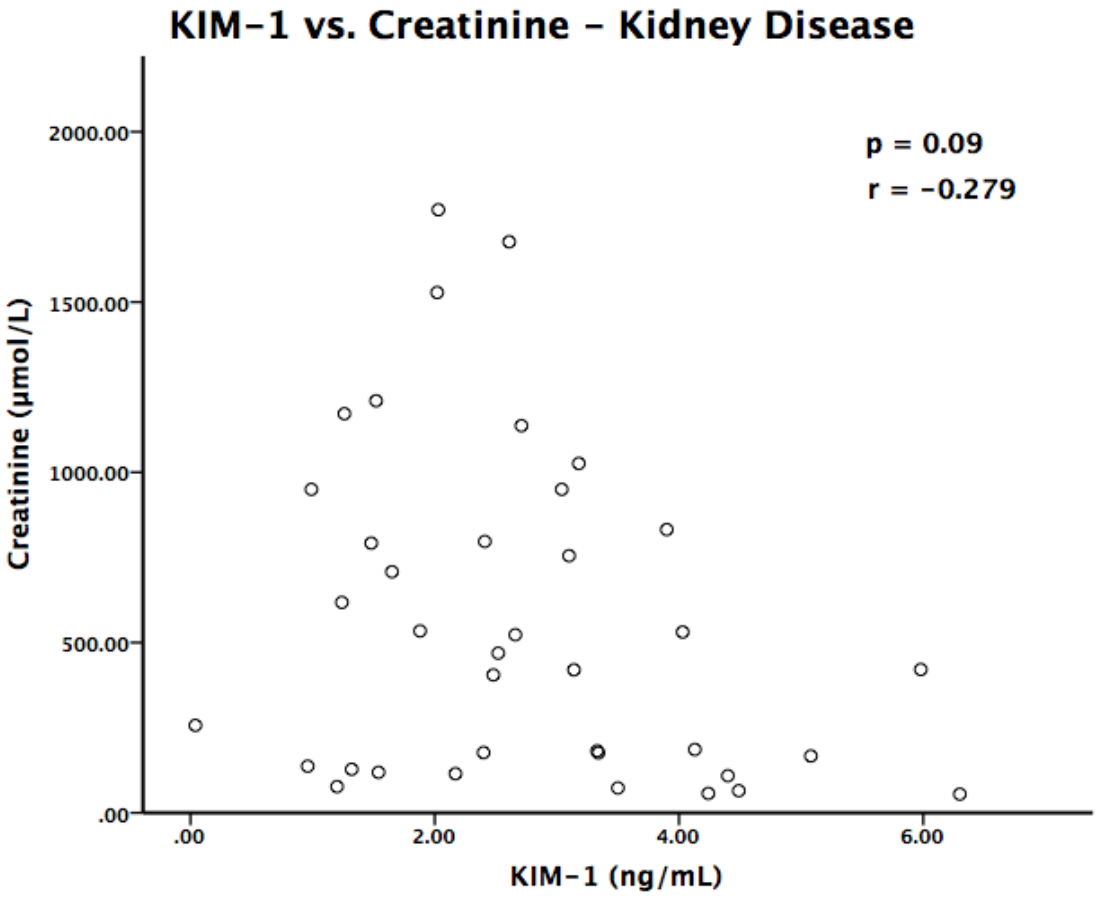

Fig 6B: KIM-1 weakly negatively correlated with Creatinine without statistical significance in hypertensive participants with p = 0.090

4.4 Pearson Correlation analysis between Microalbuminuria and Creatinine in hypertensive participants

Pearson correlation analysis of Microalbuminuria Vs Creatinine showed a positive correlation in hypertensive with statistical significance (r = 0.556, p = 0.001) (Figure 6C).

Figure 6C. Pearson Correlation analysis between Microalbuminuria and Creatinine in hypertensive participants

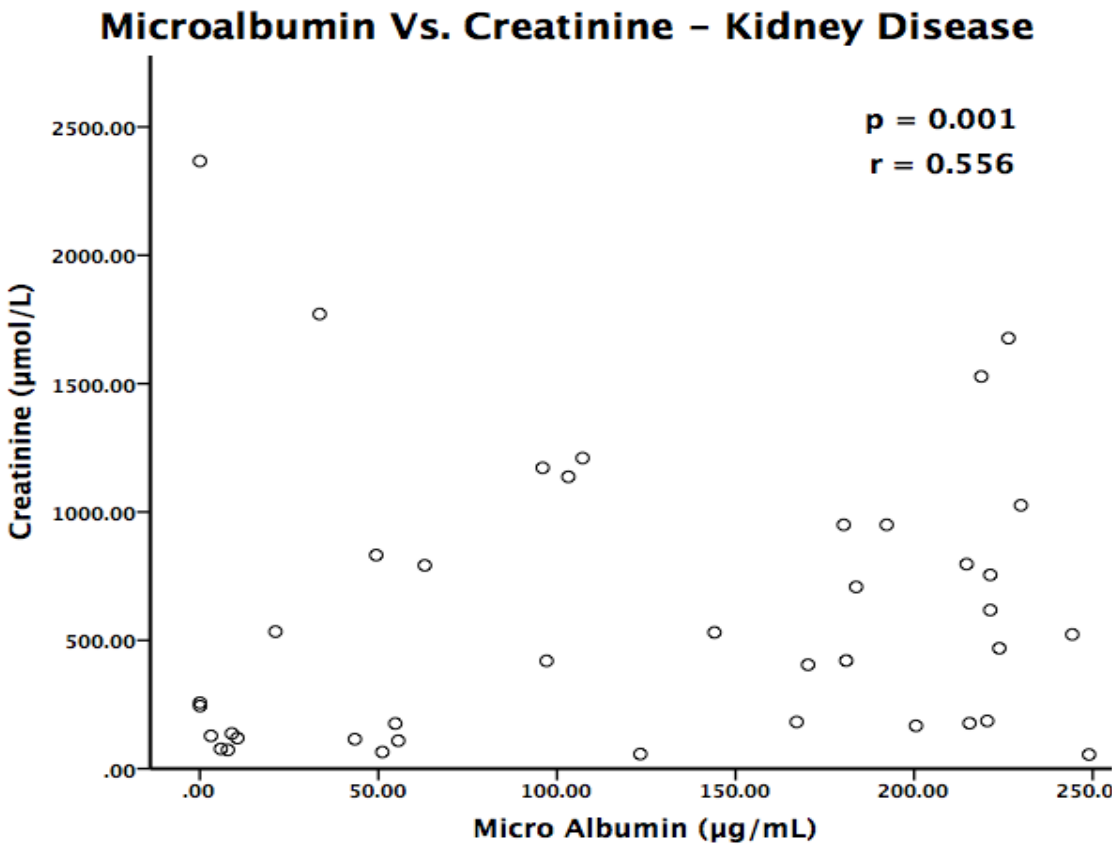

Fig 6C: Positive correlation between Microalbuminuria and Creatinine in hypertensive participants with statistical significance p = 0.001

4.5 Clinical and Biochemicals Mean Difference between hypertensives and non-hypertensives participants

There were more males (62.5%) with kidney disease than females (37.5%) in this study and the common type of kidney injury was chronic kidney disease accounting for 67.5% of all the cases with acute kidney injury having a percentage of 32.5%. There was no age difference between hypertensive participants (42.5 ± 11.2 years) and Healthy non hypertensive participants (39.2 ± 8.46 years) as analysed using independent sample t test $p = 0.6$.

Systolic and Diastolic Blood Pressure levels were higher in hypertensive individuals (135.375 ± 17.803 mmHg and 80.50 ± 12.360mmHg), compared to Healthy Control (116.154 ± 9.066 mmHg and 74.872 ± 6.437 mmHg) with statistical significance, $t(77) = 6.023$, $p < 0.001$ and $t(77) = 2.529$ $p = 0.013$ respectively (Table 1.0). Urea levels showed statistical significance between hypertensive (18.848 ± 14.716) and health controls (3.997 ± 1.716 mmol/L), $t(74) = 6.014$, $p = < 0.001$ (Table 1.0). Sodium levels were significantly lower and Potassium and Chloride were significantly higher in hypertensive participants (135.850 ± 4.775 mmol/L, 4.538 ± 0.758 mmol/L, and 106.025 ± 5.122 mmol/L respectively) to the Control Group (138.695 ± 2.162 mmol/L, 4.156 ± 0.584 mmol/L, and 102.445 ± 2.793 mmol/L), $t(74) = -3.283$; $p < 0.001$, $t(74) = 2.440$; $p = 0.016$, and $t(74) = 3.724$; $p < 0.001$ respectively (Table 1.0).

Table 1.0. Clinical and Biochemical mean difference between hypertensive and non hypertensive participants

	Hypertensive (n=40)	**Non-hypertensive(n=40)**	**T**	***df***	**p**
Age (years)	42.5 ± 11.2	39.2 ± 8.46			0.60
Systolic BP (mmHg)	135.375 ± 17.803	116.154 ± 9.0657	6.069	77	<0.001
Diastolic BP (mmHg)	80.50 ± 12.360	74.872 ± 6.437	2.547	77	0.013
Urea (mmol/L)	18.848 ± 14.716	3.997 ± 1.716	6.334	74	<0.001
Sodium (mmol/L)	135.850 ± 4.775	138.695 ± 2.162	-3.283	74	<0.001
Potassium (mmol/L)	4.538 ± 0.758	4.156 ± 0.584	2.440	74	0.016
Chloride (mmol/L)	106.025 ± 5.122	102.445 ± 2.793	3.724	74	<0.001

Hypertensive individuals with kidney injury versus non hypertensive individuals. p represents overall significant differences across groups. p-values were derived from independent sample student's *t*-test. Urea, Sodium, Potassium and Chloride were consistent with levels that are common between kidney injury patients and health individuals. Systolic and Diastolic BP consisted with levels that are common between hypertensive individuals and non hypertensive participants

4.6 KIM1 Conc. Mean Difference in Acute kidney injury versus chronic kidney disease participants

Chronic kidney disease participants had higher KIM-1 concentrations (2.736 ± 1.637ng/mL) compared to acute kidney injury participants (2.237 ± 1.204ng/mL), $t(36) = -0.968n$, $p < 0.294$ (Fig. 6A). These results did not show statistical significance showing that KIM-1was not reliable at defferitiating AKI from CKD.

Figure 7A: Mean KIM-1 concentration difference in AKI and CKD participants

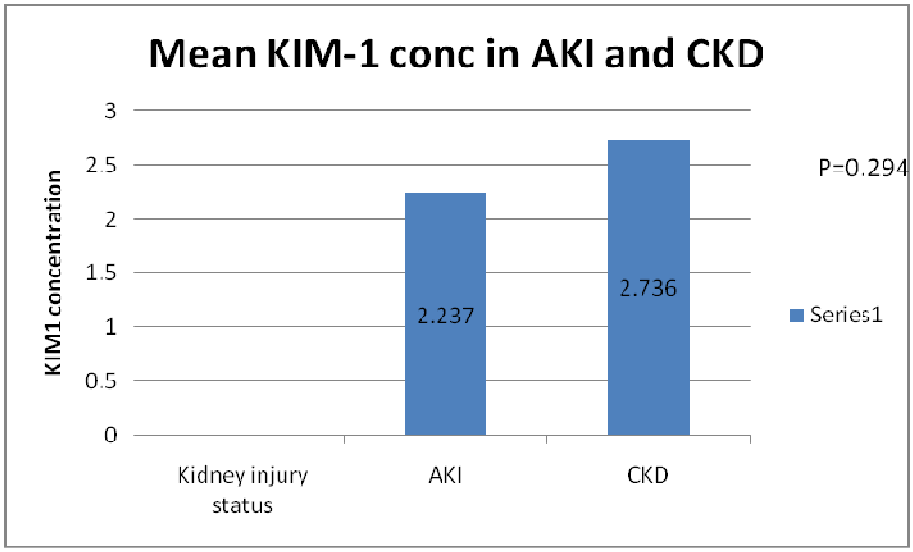

Fig 7A: KIM-1 in AKI individuals versus CKD individuals. p represents overall significant differences across groups. p-values were derived from independent sample student's *t*-test at 95% confidence interval. KIM-1 higher in CKD than in AKI with p =0.294

4.7 Microalbuminuria Conc. Mean Difference in Acute kidney injury versus chronic kidney disease participants

Chronic kidney disease participants had higher Microalbuminuria concentrations (123.204 ± 88.91µg/ml) compared to acute kidney injury participants (106.609 ± 91.765µg/ml), t(36)=-0.540 (Fig. 6B). The results did not show statistical significance showing that Microalbuminuria was not reliable at defferitiating AKI from CKD.

Figure 7B: Mean Microalbuminuria concentration in AKI and CKD participants

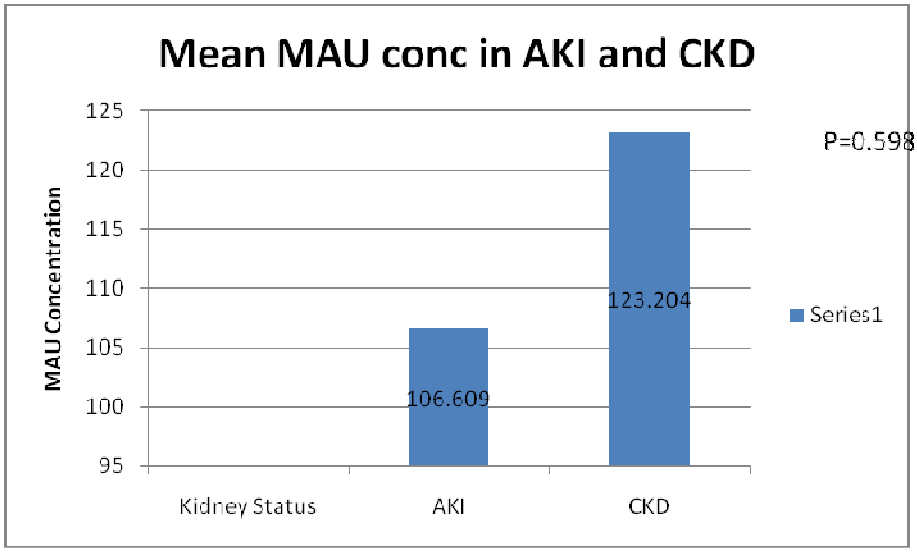

Fig 7B: Microalbuminuria in AKI individuals versus CKD individuals. p represents overall significant differences across groups. p-values were derived from independent sample student's *t*-test. Microalbuminuria were higher in CKD than in AKI with p =0.598

4.8 Creatinine Conc. Mean Difference in Acute kidney injury versus chronic kidney disease participants

Creatinine concentration were higher in AKI participants (627.465 ± 700.665 µmol/L) than in CKD participants (568.12 ± 492.747 µmol/L),$t(36) = 0.304$, p = <0.788 (Fig. 6C). These results did not show statistical significance showing that creatinine was not reliable at defferitiating AKI from CKD.

Figure 7C: Mean creatinine concentration in AKI and CKD participants

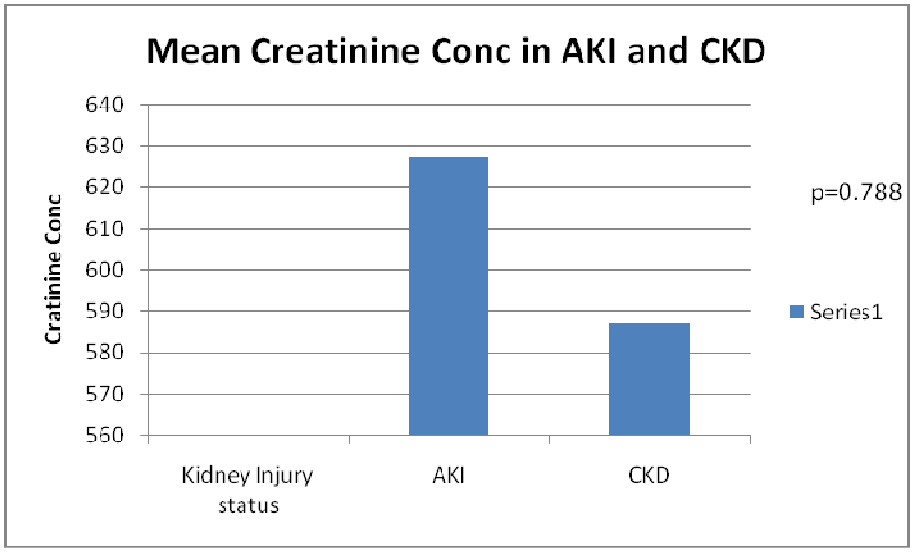

Fig 7C: Creatinine in AKI individuals versus CKD individuals. p represents overall significant differences across groups. p-values were derived from independent sample student's *t*-test. Creatinine were higher in AKI than in CKD with p =788

5.0 DISCUSSION

In this study we set out to investigate urinary KIM-1 and Microalbuminuria as possible biomarkers in the identification of both AKI and CKD in hypertensive participants.

KIM-1

The results of this study showed that there was no difference in KIM-1 concentration between hypertensive participants and non-hypertensive individuals with $p = 0.122$. These results were inconsistent with other studies that found elevated KIM-1 levels in all participating renal disease patients than controls (Vaidya *et al.,* 2008; Doi *et al*., 2009; Waanders *et al*., 2009)

The disparity in these findings could be attributed to pathophysiological changes of the kidney due to drug treatment effect (Wandaars *et al*., 2009) or it could be due to a smaller sample size in our study. This argument is also sustained by the study done by Seo *et al*. (2013) in South Korea, who demonstrated decreased KIM-1 levels after antihypertensive and renal drugs treatment. These findings could be interpreted in line with our results based on the similarity that in both studies, the participants were on renal and hypertensive drug treatment.

Waanders *et al.* (2009) in a randomized controlled trial involving Dutch participants reported that urinary KIM-1 levels decreased following antiproteinuric treatment in non-diabetic patients who had renal dysfunction (sodium restriction, Losartan, or their combination, as well as antihypertensive treatment) with $p < 0.001$, but there was no effect of therapy on creatinine levels. They suggested that urinary KIM-1 may be a useful biomarker for evaluating and predicting tubulointerstitial repair; hence an indicator of response to treatment (Waanders *et al.,* 2009; Orgrizovic, 2010)

Vaidya *et al*. (2006) and van Timmeren *et al*. (2007) reported that previously` KIM-1 was not detected in severely damaged tubules. This was because tubular KIM-1 expression is specific to ongoing tubular cell damage and differentiation in experimental and human renal disease (VanTimmeren *et al*., 2007; Seo *et al*., 2013). The decrease in urinary KIM-1 suggests that Tubular interstitial damage is ameliorated by antiproteinuric intervention (Wandaars *et al.,* 2009). In a study done by Ahmed and Hamed (2015) in Egypt, treatment of diabetic nephropathy patients showed significant decrease in KIM-1 level by 9.45% and 30.90% in male and females, respectively as compared with the diabetic nephropathy groups without treatment. This supports the potential use of KIM-1 as a biomarker for tubulointerstitial damage and repair (Seo *et al*., 2013).

The expression of KIM-1 in controls can also suggest that urinary KIM-1 excretion may be of particular value in patients with normal or mildly impaired renal function since high KIM-1 excretion i.e. above the median value predict progression of ESRD in a subgroup of patients with a serum Creatinine ≤ 135µmol/L (Parikh *et al.,* 2006). But the question still remains, what is the mechanism of KIM-1 expression in non-renal disorder, from the study controls without history of biochemical marker of kidney damage or non-renal clear cell carcinoma? Since no other organs express KIM-1 to a degree that would influence renal excretion of KIM-1 (Bonventre, 2009), the answer maybe that KIM-1 has been implicated in immune response that regulates the development of autoimmune and allergic diseases (Ibrahim *et al.,* 2010).

The other reason for the expression of KIM-1 in non hypertensive individuals could be due to the insensitivity of the markers used to define kidney disease, this is because minute or nil changes in serum creatinine may coexist with significant renal disease on account of increase tubular secretion(Ichimura *et al.,* 2008). A serum creatinine increase may remain undetected up to two to three days after kidney injury (Walkar *et al.,* 2009) while in a study done by Ibrahim *et al.* (2013) in Egypt they proved that KIM-1 could detect AKI as early as 6 hours after its occurrence before elevation of conventional markers such as serum Creatinine. A study done in the USA also concluded that KIM-1 could be expressed in urine 12 hours after initial renal ischemic insult before regeneration of epithelium and that it persists over time (Bonventre, 2009).

However, any definitive conclusion on this discrepancy should await more studies and research that are statistically powered to access the above-mentioned effects.

The probable role of KIM-1 as a biomarker of kidney injury could be explained in part by the moderate correlation between urinary KIM-1 and Microalbuminuria with statistical significance, p =0.045. These results were similar with a study involving Turkish participants that observed a positive correlation between urinary KIM-1 and Urinary albumin excretion (microalbuminuria) showing that elevated levels of urinary KIM-1 were associated with a rapid decline in renal function (Tekce *et al.,* 2014). Nahla *et al.* (2015) also observed a positive correlation between KIM-1 with Microalbuminuria, serum Creatinine and blood urea nitrogen. Its positive correlation may reflect the role of KIM -1 as a biomarker for the diagnosis of diabetic and hypertensive nephropathy (Nahla *et al.,* 2015).

However this study showed a weak negative correlation between KIM-1 and Creatinine, without statistical significance with p =0.090, the lack of statistical significance could

demonstrate that unlike KIM-1 levels, which may decrease due to treatment effect, creatinine levels may continue to increase as renal function deteriorates (Waanders *et al*., 2009).

The results were in contrast with those of Polish studies which showed a statistically significant positive correlation between KIM-1 and Creatinine (Malyszko *et al.,* 2010; Przybylowski *et al.,* 2011).

In this study there was no difference in KIM-1 levels between AKI Participants and CKD participants with p = 0.294. The lack of statistical significance could explain that KIM-1 was not reliable at differentiating AKI from CKD. The study showed that there was expression of KIM-1 in both AKI and CKD, this could explain the theory that expression of KIM-1 continues in CKD especially in areas of fibrosis (Ahmed and Hamed, 2015). Bonventre (2013) revealed that KIM-1 was localized in the apical membrane of dilated tubule in acute and chronic tubular injury. Localization of KIM-1 expression appears to be related to susceptibility of specific tubular segments of different types of injury with the S3 segment being more susceptible (Bonventre, 2013).

In CKD KIM-1 is expressed where it co localizes with areas of fibrosis and inflammation. The expression of KIM-1 in chronic and progressive kidney disease, settings without significant numbers of apoptotic cells in tubule lumen, the association of AKI with future CKD and the temporal and spatial association of KIM-1 with inflammation and fibrosis suggest that it might play a pathogenic role in linking AKI to CKD and renal fibrosis(Benjamin *et al.,* 2013).

Significantly higher levels of Creatinine ($p = < 0.001$), urea ($p = < 0.001$), potassium ($p = < 0.016$) and chloride ($p = < 0.001$) and significantly lower sodium levels with $p = < 0.001$ were observed in this study. These results were characteristic of kidney injury with a decline of renal function in the hypertensive group. More specifically, failure of the kidneys to excrete urea and creatinine, and increased loss of potassium with impaired sodium reabsorption in kidney disease (Bishop *et al.,* 2010)

Microalbuminuria

Furthermore, the hypertensive group showed statistically significant higher MAU concentration ($p = < 0.001$) than the non-hypertensive group. MAU is considered one of the most prognostically significant biomarker of kidney disease outcome and even cardiovascular and death (Poudel *et al.,* 2012; Menne *et al.,* 2012). But then, what are the implications of increased MAU expression in hypertensive participants having kidney injury? Increased MAU/ albuminuria signal an increased risk of death, cardiovascular disease and kidney disease progression (Vito *et al.,* 1999; Zacharias *et al.,* 2012). It has emerged as a strong

candidate in the prediction of renal risk in hypertensive patients, it has been proposed that this condition may signal the presence of functional and / or structural renal abnormalities that precede and predict the onset of GFR deterioration. Presence of MAU is a predictor of worse outcomes for both kidney and heart patients (Lezaic, 2010).

Our results confirms the existence of kidney injury in hypertensive participants, this could be explained by a positive correlation between MAU and Creatinine with statistical significance p = 0.001, these results were similar with those of a study by Baghel *et al.* (2014) of India which found a correlation with MAU and Creatinine reflecting impaired kidney function. The presence of MAU in hypertensive participants could indicate high risk of organ damage in these participants such as left ventricular hypertrophy, retinal vascular lesions, increased carotid artery wall thickness, stroke, glomerular hyperfiltration and peripheral atherosclerosis in some but not all patients (Pontremoli *et al.,* 1996). The risk for major cardiovascular events increases at every level of urinary albumin excretion, including levels within normal range (Tuttle *et al*., 1999).

MAU levels in our present study showed no difference between CKD and AKI participants with p = 0.598, this could mean that MAU was not reliable at differentiation of either AKI from CKD or it might be due to a greater relationship that exist between CKD and hypertension (Parving *et al.,* 1974; Sabharwal *et al.,* 2008)).

In this study there was a significant difference in blood pressure levels (systolic p = < 0.001, and diastolic p = 0.013, between hypertensive participants and non-hypertensive participants, this could explain that elevated blood pressure is considered to be a cause of kidney dysfunction (Tuttle *et al.,* 1999) , since blood pressure may differentially affect GFR and urinary albumin excretion. Increased systemic blood pressure itself may cause an increased intraglomerular pressure and, thereby, increase urinary excretion of albumin, whereas deterioration of GFR may reflect some other alterations related to hypertension as well as increased intraglomerular pressure (Waanders *et al.,* 2010). This proves the concept that urinary excretion of albumin increases with increasing blood pressure and that reduction in blood pressure itself has beneficial effects on urinary albumin excretion regardless of antihypertensive drugs used (Bakris *et al*., 2007). This was so because non hypertensive participant had significantly lower MAU levels compared to the hypertensive group.

The evaluation of Urinary albumin excretion could be regarded as a specific and inexpensive way to identify hypertensive patients at highest global risk whom more aggressive preventive strategies or additional treatment measures are advisable, although its renal prognostic value is at present uncertain (Bacanu *et al.,* 2011).

We therefore propose Physicians should be screening for MAU in patients with hypertension routinely and be as aggressive in treating this modifiable risk factor as they do blood pressure, cholesterol, or blood glucose.

6.0 CONCLUSION

The study showed that there was no difference in KIM-1 levels between hypertensive and non-hypertensive participants and MAU levels were higher in hypertensive participants than in non-hypertensive participants. The study also showed that MAU could be used as a non-invasive marker for the diagnosis of renal damage and progression in hypertensive participants; this was showed by a positive correlation between MAU and creatinine. In Zambian setup creatinine and MAU are still the biomarkers of choice for the laboratory diagnosis of kidney disease. However, the two cannot differentiate between AKI and CKD. Furthermore, findings of this study suggest that urinary KIM-1 could provide diagnostic and prognostic advantages by providing information not only for detection of kidney injury but also for predicting kidney injury reversibility following treatment

6.1. IMPLICATIONS AND RECOMMENDATIONS

Treatment options for kidney injury remain suboptimal and this is because currently it is difficult to diagnose the disease in its early stages. In order to develop new, better and more effective treatment for kidney injury, there is need for increased understanding of the pathogenetic mechanisms involved in the development of kidney injury and discovering biomarkers which are able to diagnose the disease state in its early stages as well as monitor the effects of treatment so that therapy may target the correct mechanism or mechanisms involved in the disease process before the disease becomes irreversible. Findings of this study indicate that more research needs to be carried out on KIM-1and MAU as important biomarkers that could provide diagnostic, prognostic and therapeutic advantages by providing information on the diagnosis of the disease.

6.2. LIMITATIONS / WEAKNESSES

The study was limited to hypertensive participants who had already been diagnosed with kidney injury hence treatment options could have confounded the findings this was because, once a patient is diagnosed with kidney injury, he or she is put on treatment which affects most of the biomarkers.

Previous surgery, cancer, haematuria, liver disease, urinary tract infection, acute allergies, exercises and any acute and chronic inflammatory conditions were not determined but

assessed by means of checking participant's hospital file records; these could have confounded the research findings.

A third group of individuals with kidney injury (in its early stages) who had not started treatment would have been included in the study to check for KIM-1 levels in early stages to determine how long after kidney injury does tubular epithelial cells start to excrete KIM-1 in urine. However, the researcher did not have necessary tools and finances for determining kidney injury in its early stage, the researcher relied on the already diagnosed kidney injury patients. The other limitation of the study was failure to repeat the MAU test, it is important to repeat the MAU test because MAU levels varies widely in urine, for example it can vary with posture, exercise and blood pressure.

6.3 FUTURE DIRECTION

With respect to the above considerations, more supportive and definitive investigation is required. A similar study need to be carried out in newly admitted and diagnosed AKI patients who are not yet on medication.

There is also need for the patients to be followed up over a long time to give provision for repeating microalbuminuria levels which is very important in order to determine MAU levels of an individual

Since some of our conclusions are merely theoretical, the effect of therapy/ medication on KIM-1 and MAU levels and the expression of KIM-1 in study controls should further be investigated maybe by doing large longitudinal studies which involves follow up or, it may be necessary to conduct animal model studies in order to conclusively elucidate whether the development and/or the progression of kidney damage or cardiovascular events can be prevented or reversed by the early detection of the same using KIM-1 or MAU. And also to determine how long after injury do tubular epithelial cell express KIM-1 on their surface.

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8.0 APPENDECES

Appendix 1

Table 2.0 Hypertensive participants Raw Data

#	SEX	AGE Years	BP (mmHg)	KI STATUS	CREATININE (µmol/l)	UREA mmol/l	Na mmol/l	K mmol/l	CHLORIDE mmol/l	KIM-1 ng/mL	MAU µg/mL
1	M	28	140/80	AKI	2367	63.6	135	6.2	107	**0.23**	**0.01**
2	M	53	130/70	CKD	182	11.9	135	4.7	105	**3.33**	**167.11**
3	M	37	170/90	CKD	797	21.4	132	4.8	111	**2.41**	**214.70**
4	F	33	140/90	AKI	708	20.8	136	5.1	113	**1.65**	**183.75**
5	M	43	140/70	CKD	244	7.5	135	3.9	101	**0.18**	**0.06**
6	F	51	140/90	CKD	1528	35.7	136	6.4	109	**2.02**	**218.81**
7	M	36	140/90	AKI	77	2.4	135	3.6	103	**1.20**	**5.87**
8	M	50	150/90	CKD	1771	49.9	133	4.6	105	**2.03**	**33.53**
9	M	72	150/70	CKD	950	39.8	135	5.8	113	**3.04**	**180.29**
10	M	38	120/70	AKI	57	15.1	132	3.9	104	**4.24**	**123.33**
11	F	46	130/90	CKD	65	4.2	139	4.1	108	**4.49**	**51.11**
12	M	41	120/70	CKD	421	15.7	139	4.6	110	**5.98**	**180.98**
13	M	42	139/82	CKD	1026	34	132	4.4	110	**3.18**	**229.89**
14	M	45	120/70	CKD	792	17.1	135	5.4	114	**1.48**	**62.98**
15	F	48	110/70	CKD	73	3.7	137	3.8	108	**3.50**	**7.86**
16	M	23	140/70	CKD	1137	13.6	132	4.5	103	**2.71**	**103.18**
17	M	43	130/60	AKI	128	6.9	137	4.9	105	**1.32**	**3.11**
18	M	56	140/90	AKI	950	34.7	135	6	110	**0.99**	**192.31**
19	F	36	130/70	AKI	469	13.7	133	4	104	**2.52**	**223.86**
20	F	18	100/60	CKD	55	2.2	138	4.3	106	**6.30**	**248.97**
21	M	65	130/80	CKD	257	13.2	138	3.5	107	**0.04**	**0.04**
22	F	40	110/60	AKI	177	14	140	3.7	109	**2.40**	**215.52**
23	F	60	130/70	CKD	523	27.7	137	4.9	106	**2.66**	**244.28**
24	M	41	150/90	CKD	137	5.6	137	4	104	**0.96**	**8.95**
25	M	67	160/90	CKD	186	14.8	144	4.9	98	**4.13**	**220.48**
26	F	41	120/80	CKD	109	7.6	138	4.1	107	**4.40**	**55.56**
27	F	29	130/70	AKI	420	14	123	3.4	105	**3.14**	**97.09**
28	F	70	160/100	CKD	115	5.9	140	4	109	**2.17**	**43.41**
29	M	30	120/90	CKD	405	15.8	134	5.9	99	**2.48**	**170.31**
30	M	59	160/90	CKD	755	26.3	139	5	108	**3.10**	**221.32**
31	F	47	160/90	AKI	1677	48.8	128	4.4	91	**2.61**	**226.42**
32	F	24	140/90	CKD	531	45.6	149	4.7	97	**4.03**	**144.11**

33	M	26	110/70	AKI	176	9.1	138	3.7	108	**3.34**	**54.73**
34	M	37	100/80	AKI	119	8.8	126	4.1	100	**1.54**	**10.53**
35	M	65	136/88	CKD	1172	9.3	130	4.8	104	**1.26**	**95.99**
36	M	28	170/120	AKI	832	9.7	141	3.9	109	**3.90**	**49.39**
37	F	39	160/90	CKD	1210	8.9	137	4.5	105	**1.52**	**107.17**
38	M	52	130/70	CKD	534	14.2	143	3.7	103	**1.88**	**21.18**
39	M	19	120/80	CKD	618	25.6	140	4.8	120	**1.24**	**221.32**
40	F	22	140/80	CKD	167	15.1	131	4.5	103	**5.08**	**200.52**

Appendix 2

Table 3.0. Non-hypertensive participants Raw Data

#	Sex	Age Years	BP mmHg	Creatinine µmol/l	Urea mmol/l	Na mmol/l	K mmol/l	Chloride mmol/l	KIM-1 ng/ml	MAU µg/ml
41	M	33	120/70	78	3.9	137	4.3	96	**4.00**	**13.03**
42	M	24	110/80	86	3.4	140	4	94	**1.76**	**9.29**
43	M	32	120/80	62	2.6	140	4.2	98	**4.22**	**45.44**
44	M	20	130/80	60	2.5	142	3.1	99	**1.61**	**16.61**
45	F	32	120/70	55	4	142	3.4	100	**3.44**	**2.91**
46*	M	55	140/80	111	11.3	138	3.6	97	**3.64**	**95.27**
47	M	26	120/70	80	3.2	139	3.6	101	**2.21**	**3.46**
48	F	37	120/80	59	3.8	139	4.1	105	**2.43**	**2.60**
49	F	23	110/80	59	2.5	140	4.1	100	**1.50**	**80.61**
50	M	25	110/80	90	5.7	143	3.7	102	**2.65**	**8.07**
51	F	19	120/80	46	1.9	140	3.5	102	**1.89**	**2.43**
52	M	44	110/60	74	5.3	142	4	98	**3.73**	**9.15**
53	M	29	120/70	100	3.2	140	4.5	106	**2.94**	**9.15**
54	M	30	110/80	78	4.3	142	5	101	**1.81**	**22.08**
55	M	34	120/80	101	3.7	139	5.1	102	**3.21**	**2.64**
56	M	18	100/70	110	4.5	139	5.3	104	**3.24**	**11.71**
57	M	40	110/80	99	5	138	4.7	105	**3.09**	**1.44**
58	F	25	120/70	53	4.2	138	3.9	105	**4.57**	**6.83**
59	F	19	100/80	64	3.4	137	3.7	106	**3.06**	**7.43**
60	F	18	130/70	54	5.7	138	4.4	102	**5.98**	**62.03**
61	M	31	110/80	90	3.3	140	4.3	103	**4.24**	**4.45**
62	M	28	120/80	61	2.4	138	4.3	102	**3.68**	**10.90**
63	F	25	120/80	52	3.5	138	3.8	105	**4.41**	**8.26**
64	M	33	110/70	50	2	137	3.8	103	**4.30**	**16.93**
65	M	38	120/70	77	4.6	134	3.8	104	**5.24**	**13.90**
66	M	30	100/70	78	4.5	138	3.8	105	**3.88**	**8.23**
67	M	24	120/80	61	2.1	135	5.5	101	**3.04**	**11.67**

68	M	40	130/80	93	5.2	134	5.7	104	**2.66**	**9.84**
69	F	20	110/60	57	3.9	138	4.6	103	**2.92**	**8.42**
70	M	36	110/80	82	2.9	138	4.2	102	**2.58**	**0.35**
71	F	18	120/80	53	3.8	135	4.1	105	**2.14**	**29.03**
72	M	33	120/70	71	6.2	137	4.2	101	**5.47**	**14.11**
73	F	30	100/70	62	2.2	139	3.9	104	**4.26**	**17.52**
74	M	32	120/70	77	2.5	138	3.8	106	**1.91**	**3.40**
75	M	21	110/80	67	4.8	139	3.9	104	Missing	**18.90**
76	F	20	120/80	60	3.7	138	3.9	105	Missing	**5.38**
77	M	25	110/60	73	6.1	141	4.1	104	Missing	**14.43**
78	F	28	110/80	68	2.7	132	3.4	99	Missing	**3.81**
79	F	29	130/70	72	3.0	138	4.7	105	Missing	**7.21**
80	F	23	120/70	59	4.0	136	3.5	97	Missing	**20.39**

* Increased urea levels could be attributed to the fact that urea levels is affected by the individual hydration status, protein diet as well as some medication such as tetracycline. These factors could have lead to the participant urea to be high other than renal damage because from the information obtained from the participant file and the interviews administered questionnaire the participant had no renal disease

THE UNIVERSITY OF ZAMBIA

BIOMEDICAL RESEARCH ETHICS COMMITTEE

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Assurance No. FWA00000338
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23rd September, 2014.

Our Ref: 003-08-14.

Ms. Mildred Zulu,
University of Zambia,
School of Medicine,
Department of Pathology and Microbiology,
P. O Box 50110,
Lusaka.

Dear Ms. Zulu,

RE: RESUBMITTED RESEARCH PROPOSAL: **"ESTIMATION OF KIDNEY INJURY-MOLECULE-1 LEVELS AS A MARKER OF KIDNEY INJURY IN HYPERTENSIVE PATIENTS AT THE UNIVERSITY TEACHING HOSPITAL OUTPATIENT MEDICAL CLINIC, LUSAKA" (REF. No. 003-08-14)**

The above-mentioned research proposal was presented to the Biomedical Research Ethics Committee on 22nd September, 2014. The proposal is approved.

CONDITIONS:

- This approval is based strictly on your submitted proposal. Should there be need for you to modify or change the study design or methodology, you will need to seek clearance from the Research Ethics Committee.
- If you have need for further clarification please consult this office. Please note that it is mandatory that you submit a detailed progress report of your study to this Committee every six months and a final copy of your report at the end of the study.
- Any serious adverse events must be reported at once to this Committee.
- Please note that when your approval expires you may need to request for renewal. The request should be accompanied by a Progress Report (Progress Report Forms can be obtained from the Secretariat).
- **Ensure that a final copy of the results is submitted to this Committee.**

Yours sincerely,

Dr. J.C. Munthali
CHAIRPERSON

Date of approval: 23rd September, 2014. **Date of expiry:** 22nd September, 2015.

Appendix 4: List of Publications and Presentations

The following manuscript, based on this dissertation, has been submitted for publication in *African Health Sciences.*

Mildred Zulu, Musalula Sinkala, Chisanga Chileshe, Timothy Kantenga and Trevor Kaile (2015). Kidney Injury Molecule-1 and Microalbuminuria in Renal Hypertensive Zambians: Biomarkers of Kidney Injury

Part of this work has been presented at the following scientific meetings

1. Mildred Zulu and Trevor Kaile (2015). Estimation of Kidney Injury Molecule-1 and Microalbuminuria levels in Renal - Hypertensive patients at the University Teaching Hospital, Lusaka, Zambia Oral Presentation at the University of Zambia Post graduate seminar week, 13th – 17th July, 2015.
2. Mildred Zulu and Trevor Kaile (2015). Estimation of Kidney Injury Molecule-1 and Microalbuminuria levels in Renal - Hypertensive patients at the University Teaching Hospital, Lusaka, Zambia Poster Presentation at the University of Zambia Post graduate seminar week, 13th – 17th July, 2015.
3. Mildred Zulu and Trevor Kaile (2015). Estimation of Kidney Injury Molecule-1 and Microalbuminuria levels in Hypertensive patients at the University Teaching Hospital, Lusaka, Zambia postgraduate defence, 9th October, 2015