ASSESSMENT OF SERUM CYSTATIN C, CREATININE AND ESTIMATED GLOMERULAR FILTRATION RATE IN PATIENTS WITH CHRONIC KIDNEY DISEASE IN ZARIA, NIGERIA

BY

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BY

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DECLARATION

I declare that the work in this Dissertationentitled**Assessment of serum cystatin C, creatinine and estimated Glomerular Filtration Rate in patients with chronic kidney disease in Zaria, Nigeria,**has been carried out by me in the Department of Chemical Pathology,Ahmadu Bello University, Zaria. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this project was previously presented for another degree or diploma at this or any other institution.

RabiuAdamu……. …….

(Signature) (Date)

CERTIFICATION

This dissertation entitled **ASSESSMENT OF SERUM CYSTATIN C, CREATININE AND ESTIMATED GLOMERULAR FILTRATION RATE IN PATIENTS WITH CHRONIC KIDNEY DISEASE IN ZARIA, NIGERIA** by RabiuADAMUmeets the regulations governing the award of thedegree of MSc Chemical Pathology of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

The limitations of serum creatinine as a marker of impairment in GFR have led to continuing search for a better marker. Most studies have shown that serum cystatin C may be a more sensitive indicator of glomerular filtration rate (GFR) than serum creatinine in the clinical setting. This study was designed to evaluate serum cystatin C,creatinineand estimated GFR (eGFR)in Chronic Kidney Disease (CKD) patients.

One hundred and fourteen (114) CKD Patients (comprising 59 males and 55 females) attending Nephrology Clinic at ABUTH and apparently healthy age and sex matched controls were recruited for the study. Serum creatinine and cystatin C were assayed by the Jaffe kinetic and immunoturbidimetric methods respectively. Estimated GFR was calculated usingthe Cockcroft-Gault (C-G) formula. Serum creatinine, cystatin C and Estimated GFR (eGFR) in patients and controls were comparedusing the t-test. Relationships between serum cystatin C, creatinine and estimated GFR were analysed using the Pearson's correlation.

In this study,serumcystatin C and creatinine were significantly higher($p<0.0001$)and estimated GFR significantly lower ($p<0.0001$) in patients than the control subjects.Cystatin C was better correlated with Estimated GFR ($r =-0.708; p<0.0001$) than creatinine ($r =-0.694; p<0.0001$). Mean cystatin C concentration showed step-by-step statistically significant increase as GFR decreases, allowing very early detection of reduction in renal function.

The findings from this study showed that serum cystatin C is a better marker compared with serum creatininein evaluating GFR particularly for detecting very early reduction of renal function. Use of cystatin C to assess renal function will optimize early detection,treatment and monitoring strategies forpatients with CKD.

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ABBREVIATIONS/SYMBOLS

A Absorbance

A_{std} Absorbance of standard

ABUTH Ahmadu Bello University Teaching Hospital

ACEIs Angiotensin converting enzyme inhibitors

ARBs Angiotensin II receptor blockers

α Alpha

β Beta

BP Blood pressure

BTP Beta trace protein

CDC Centre for Disease Control

CRF Chronic renal failure

CKD Chronic kidney disease

C Concentration

C_{std} Concentration of standard

Cr Creatinine

CrCl Creatinine Clearance

CVD Cardiovascular disease

^{o}C Degree Celsius

δ Delta

DM Diabetes mellitus

e.g For example

eGFR EstimatedGlomerular filtration rate

ELISA Enzyme-linked immunosorbent assay

ESRD End stage renal disease

ESRF End stage renal failure

et al and others

G Gauge

g Gram

GFR Glomerular filtration rate

> Greater than

< Less than

≥ Greater than or equal to

≤ Less than or equal to

H Hydrogen

HIV Human immunodeficiency virus

HIVAN HIV- associated nephropathy

K/DOQI Kidney Disease Outcome Quality Initiative

kg Kilogram

kg/m2 Kilogram per metre square

µL Microlitre

µmol Micromole

µmol/L Micromole per litre

MDRD Modification of Diet in Renal Disease

ml Milliliter

ml/min Milliliter per minute

mmol Millimole

mmol/L Millimole per litre

min Minute

ng Nanogram

% Per cent

± Plus or minus

rpm Revolution per minute

RRT Renal replacement therapy

SPSS Statistical package for the social sciences

std Standard

USAUnited States of America

WHO World Health Organisation

Wt Weight

yr Year

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

Chronic kidney disease (CKD) is defined by the occurrence of kidney damage or glomerular filtration rate (GFR) <60 mL/min/1.73 m^2 for ≥3 months. According to the definition by the Kidney Disease Outcome Quality Initiative (K/DOQI) (2002), the presence of CKD should be established base on the occurrence of kidney damage and the level of kidney function, regardless of the specific diagnosis of diseases and conditions causing the damage.Disease staging is based on the GFR (Table 1.1). Chronic kidney disease is characterized by a gradual and permanent loss of kidney function that worsens as it progresses from stages 1 to 5 (National Kidney Foundation. K/DOQI 2002).

The global increase in the incidence and prevalence of CKD is being driven by the global increase in the prevalence of diabetes mellitus, hypertension, obesity, and aging.The prevalence of CKD increases exponentially with age, and we can expect numbers to rise as the population continues to age and the prevalence of type II diabetes increases (Stevens *et al*, 2007). The World Health Report (2003) and Global Burden of Disease project reports show that diseases of the kidney and urinary tract contribute to the global burden of diseases with approximately 847,000 deaths every year and 15, 214,000 disability-adjusted life years (WHO, 2003). Community surveys have shown that the number of people with end-stage kidney disease (ESRD) is just the tip of the "CKD iceberg" (Olugbenga*et al*, 2010).

More than 10% of people, or more than 20 million, aged 20 years or older in the United States have CKD.More than 35% of people aged 20 years or older with diabetes have CKD and more than 20% of people aged 20 years or older with hypertension have CKD (CDC, 2010).

Table 1.1 Stages of CKD by GFR (National Kidney Foundation. K/DOQI 2002).

Stage	Description	GFR ml/min/m2
1	Kidney damage with normal or increased *GFR*	>90
2	Kidney damage with mild decrease GFR	60-89
3	Moderate decrease GFR	30-59
4	Severe decrease GFR	15-29
5	Kidney failure	<15

Although middle-aged and elderly populations are predominantly affected in developed countries, in sub-Saharan Africa, CKD mainly affects young adults in their economically productive years, with hypertension and infection-related chronic glomerulonephritis as the major causes. Morbidity and mortality are high because most affected individuals cannot access renal replacement therapy (Arogundade and Barsoum, 2008).In Nigeria the peak prevalence of CKD is between the third and fifth decade of life, thus contributing to manpower shortage and economic waste **(**Alebiosu and Ayodele, 2005).

Hypertension, glomerulonephritis (GN), sickle cell disease, quartan malaria nephropathy, urinary tract schistosomiasis and other parasite-related forms of chronic glomerulonephritis are known to contribute significantly to the incidence of CRF in Nigeria (Odubanjo*et al*, 2011). As in the other parts of the world, diabetic nephropathy appears to be of increasing importance in the causation of ESRD in Nigeria (Odubanjo*et al*, 2011).

Like other developing countries of the world, there is no reliable statistics assessing the prevalence of kidney diseases in Nigeria **(**Alebiosu and Ayodele, 2005). The prevalence of chronic renal failure (CRF) has been shown to differ in various parts of Nigeria (Odubanjo et *al*, 2011).In a 10 year retrospective study in a University Teaching Hospital in South West Nigeria,Olutayo*et al* (2006) reported a 3.6% frequency of CKD in the population.Abioye-Kuteyi*et al* (1999) reported a prevalence of 19.9% of undetected renal diseases in a rural populace in Nigeria. In a study at Family Practice Clinic, Afolabi*et al* (2009) reported that 10.4% of the subjects had a persistent low GFR, compared to 12.4% with persistent albuminuria. Akinsola*et al* (1989) reported that renal failure constituted 8% of hospital admissions while Nwankwo et *al* (2006) reported an incidence of 45.5% of impaired kidney function among hospitalized hypertensive patients in Maiduguri, Nigeria.

In the early stages of CKD, symptoms may not be present and about 20% of patients can have CKD for a number of years. This is important, for example, in those with undiagnosed Type 2 Diabetes who when eventually diagnosed may present with established kidney damage (Tomson and Udayaraj, 2007).

The rate of progression of CKD, irrespective of underlying cause is dependent on both non-modifiable factors such as age, sex, race, the level of kidney function at diagnosis and modifiable characteristics including proteinuria, level of blood pressure control and smoking (Michael *et al*, 2006).Chronic kidney disease is associated with an eight to tenfold increase in cardiovascular mortality and is a risk multiplier in patients with diabetes and hypertension (Couser*et al*, 2011**)**. Established kidney failure with GFR <15 mL/min/1.73 m^2 is referred to as end-stage renal disease (ESRD) and requires renal replacement therapy in form of dialysis or kidney transplant (National Kidney Foundation. K/DOQI, 2002).End-stage renal disease disproportionately affects people in developing countries as well as ethnic and racial minorities in the United States (Olutayo*et al*, 2006).

The economic consequences of CKD can be in the form of direct loss of gross domestic product as a result of ill health, losses due to household financing of care, changes in consumption patterns and welfare costs as well as the financial cost incurred in managing patients with CKD and ESRD (Ulasi and Ijoma, 2010; Vivekanand, 2012). Early identification of patients with CKD may allow implementation of multiple risk factor intervention strategies aimed at reducing morbidity, mortality, and disease progression. However, this is yet to be proven and we are yet to identify a comprehensive, cost-effective means of identifying patients with CKD at an early stage (Stevens et al, 2007; Bosan, 2007).

1.2 STATEMENT OF THE PROBLEM

Even though CKD is emerging in the 21st century as a global public health issue, there is no satisfactory marker yet for early diagnosis and optimal monitoring of patients with this disease.

The current marker, serum creatinine, used in assessing kidney dysfunction in our centre is not accurate for rapidly changing (deteriorating) GFR; it is unreliable in acutely oliguric and anuric patients. It varies with age, gender and lean muscle mass, and its serum concentration start to rise only when substantial renal function is already lost.

1.3 JUSTIFICATION

Serum cystatin C, unlike serum creatinine, is not affected by age, sex, muscle mass and high protein diet. Serum cystatin C may offer a better estimate of GFR than serum creatinine and is devoid of the limitations inherent in serum creatinine assays. There is paucity of data on the serum level of cystatin C in the assessment of renal function in our environment.

1.4 AIM AND OBJECTIVES

1.4.1 Aim

The study is aimed at assessing serum cystatin C, creatinine and eGFR in CKD patients in Zaria, Nigeria.

1.4.2 Objectives

1) To measure serum cystatin C and creatinine concentrations in CKD patients attending Nephrology clinic in ABUTH, Zaria, and control subjects.
2) To compare serum cystatin C, creatinine and estimated GFR in CKD patients with that of the control subjects.
3) To determine the relationship between serum cystatin C, creatinine and estimated GFR in CKD patients attending Nephrology Clinic in ABUTH, Zaria.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 THE KIDNEY

The kidneys are a paired organ system located in the retroperitoneal space. The adult kidney is about 12cm long and weigh about 150g in men and 135g in women. They extend from the lower part of the 11th thoracic vertebra to the upper portion of the 3rd lumber vertebra, with the right kidney situated slightly lower than the left (Michael, 2006).

In the majority of cases, each kidney receives its blood supply from a single renal artery derived from the abdominal aorta, with the venous return along a renal vein that drains into the vena cava. The renal lymphatic drainage includes fine lymphatics in the glomerulus, some in close proximity to the juxtaglomerular apparatus, which are associated with removal of materials from the mesangial cells (Robert and Barry, 2005).

The kidneys have both sympathetic and parasympathetic nerve supply whose main function appears to be predominantly associated with vasomotor activity (Michael, 2006).

Approximately 25% of the cardiac output or 1200 ml of blood per minute is received by the kidneys. One litre of urine is the end product of more than 1,000 litres of circulating blood processed through the kidneys (Robert and Barry, 2005).

The Nephron (Fig 2.1) is the functional unit of the kidney. Each kidney in the human contains about one million nephrons, each capable of forming urine. The kidney cannot regenerate new nephrons. Therefore, with renal injury, disease, or normal aging, there is a gradual decrease in nephron number. Each nephron contains a tuft of glomerular capillaries called the glomerulus, through which large amounts of fluid are filtered from the blood, and a long tubule in which the filtered fluid is converted into urine on its way to the pelvis of the kidney. The glomerulus contains a network of branching and anastomosing glomerular capillaries that are covered by epithelial cells, and the total glomerulus is encased in

Bowman’s capsule. Fluid filtered from the glomerular capillaries flows into Bowman’s capsule and then into the proximal tubule, which lies in the cortex of the kidney(Robert and Barry, 2005).

From the proximal tubule, fluid flows into the loop of Henle, which dips into the renal medulla. Each loop consists of a descending and an ascending limb. The walls of the descending limb and the lower end of the ascending limb are very thin and therefore are called the thin segment of the loop of Henle. After the ascending limb of the loop has returned part-way back to the cortex, its wall becomes much thicker, and it is referred to as the thick segment of the ascending limb. At the end of the thick ascending limb is a short segment, which is actually a plaque in its wall, known as the macula densa which plays an important role in controlling nephron function. Beyond the macula densa, fluid enters the distal tubule, which, like the proximal tubule, lies in the renal cortex. This is followed by the connecting tubule and the cortical collecting tubule, which lead to the cortical collecting duct. The initial parts of 8 to 10 cortical collecting ducts join to form a single larger collecting duct that runs downward into the medulla and becomes the medullary collecting duct. The collecting ducts merge to form progressively larger ducts that eventually empty into the renal pelvis through the tips of the renal papillae. In each kidney, there are about 250 of the very large collecting ducts, each of which collects urine from about 4,000 nephrons (Arthur and John, 2006).

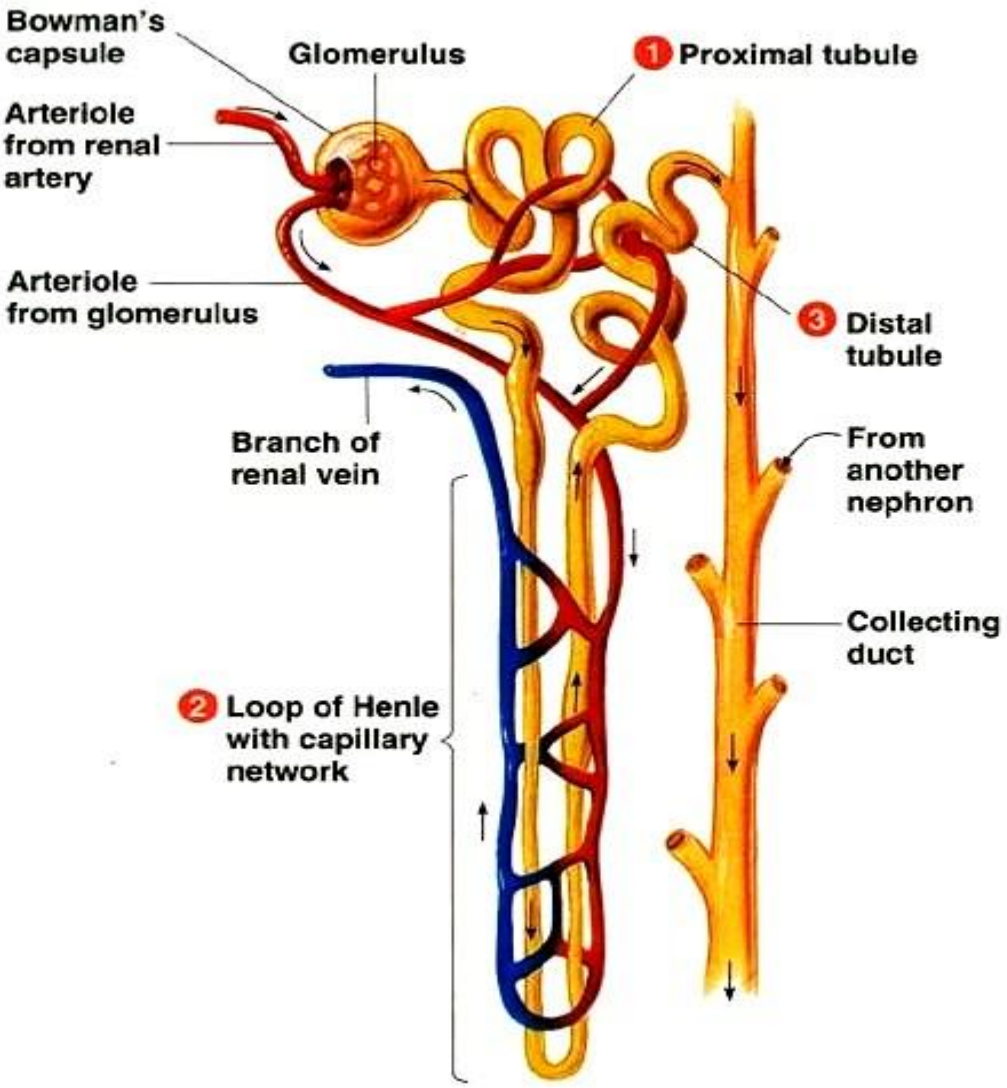

Figure 2.1.The nephron (www.biology4isc.weebly.com)

2.1.1 Functions of the Kidney

The kidneys are vital excretory organs and are central to fluid, electrolyte and acid-base homeostasis in humans. Damage of the kidneys has serious implications for systemic functions, growth and existence (Afolabi*et al*, 2009). They form urine in which the potentially toxic waste products of metabolism are excreted. The functions of the kidney therefore include;

2.1.1.1 Excretion of Metabolic Waste Products, Foreign Chemicals, Drugs, and Hormone Metabolites

The kidneys are the primary means for eliminating waste products of metabolism that are no longer needed by the body. These products include urea (from the metabolism of amino acids), creatinine(from muscle creatine), uricacid(from nucleic acids), end products of haemoglobin breakdown (such as bilirubin), and metabolites of various hormones. The kidneys also eliminate most toxins and other foreign substances that are either produced by the body or ingested, such as pesticides, drugs, and food additives.

2.1.1.2 Regulation of Water and Electrolyte Balances

For maintenance of homeostasis, excretion of water and electrolytes must precisely match intake. The capacity of the kidneys to alter sodium excretion in response to changes in sodium intake is enormous. Studies have shown that in many people, sodium intake can be increased to 1500 mmols/day (more than 10 times normal) or decreased to 10 mmols/day (less than one tenth normal) with relatively small changes in extracellular fluid volume or plasma sodium concentration *(*Arthur and John, 2006)**.** This is also true for water and for most other electrolytes, such as chloride, potassium, calcium, hydrogen, magnesium, and phosphate ions.

2.1.1.3 Regulation of Arterial Pressure

The kidneys play a dominant role in long-term regulation of arterial pressure by excreting variable amounts of sodium and water. The kidneys also contribute to short-term arterial pressure regulation by secreting vasoactive factors or substances, such as renin, that lead to the formation of vasoactive products (e.g., angiotensin II).

2.1.1.4 Regulation of Acid-Base Balance

The kidneys contribute to acid-base regulation, along with the lungs and body fluid buffers, by excreting acids and by regulating the body fluid buffer stores. The kidneys are the only means of eliminating from the body certain types of acids, such as sulfuric acid and phosphoric acid, generated by the metabolism of proteins.

2.1.1.5 Regulation of Erythrocyte Production

The kidneys secrete erythropoietin, which stimulates the production of red blood cells.

2.1.1.6 Regulation of 1,25–Dihydroxyvitamin D3 Production

The kidneys activates vitamin D to the active form vitamin D, 1,25-dihydroxyvitamin D3 (calcitriol), by hydroxylating this vitamin at the "number 1" position. Calcitriol is essential for normal calcium deposition in bone and calcium reabsorption by the gastrointestinal tract.

2.1.1.7 Glucose Synthesis

The kidneys synthesize glucose from amino acids and other precursors during prolonged fasting, a process referred to as gluconeogenesis *(*Arthur and John, 2006**).**

2.2 CHRONIC KIDNEY DISEASE

Chronic kidney disease has been defined as decreased kidney function and/ or kidney damage persistent for at least 3 months. Kidney dysfunction is indicated by a glomerular

filtration rate (GFR) of less than 60 mL/min/1.73 m^2, while kidney damage most frequently is manifested as increased urinary albumin excretion (National Kidney Foundation. K/DOQI, 2002). Within this framework, CKD has been categorized into five stages:

Stage 1: Kidney damage with GFR ≥90 mL/min/ 1.73 m^2.

Stage 2: Kidney damage with GFR 60–89 mL/min/ 1.73 m^2.

Stage 3: GFR 30–59 mL/min/1.73 m^2 regardless of kidney damage.

Stage 4: GFR 15–29 mL/min/1.73 m^2 regardless of kidney damage.

Stage 5: GFR <15 mL/min/1.73 m^2 regardless of kidney damage, or kidney failure treated by dialysis or transplantation.

2.2.1 Aetiopathogenesis of CKD

Infrequently, CKD is caused by primary kidney disease (e.g., glomerular diseases, tubulointerstitial diseases, obstruction, and polycystic kidney disease). But in the vast majority of cases, it is associated with other medical conditions, such as diabetes and hypertension (Effective Health Care Program, 2012). Whatever the underlying etiology, the destruction of renal mass with irreversible sclerosis and loss of nephrons leads to a progressive decline in GFR. In a 13 year (1990-2003) retrospective study by Ulasi and Ijoma (2010) in a University Teaching Hospital in South-East Nigeria, the primary cause of renal disease could not be determined in 56% of the 1,538 patients; however in those whose primary cause of renal disease was known, hypertension was most prevalent (17.2%). Thirty two (4.7%) patients had confirmed HIV while 29 (4.3%) were documented to have HIV-associated nephropathy (HIVAN). The 29 patients with HIVAN constituted 13% of the patients with chronic glomerulonephritis. In 22 (3.3%) -patients, history of ingestion of a nephrotoxic agent and urinalysis showing leucocyturia with eosinophilia among other

findings were suggestive of nephrotoxic damage. Type 2 DM was noted in 147 (80.8%) while 35 (19.2%) had type 1 DM.

2.2.2 Clinical Features of CKD

A significant number of people may have chronic kidney disease without being aware of it. Symptoms become obvious when the glomerular filtration rate (GFR) is only 20 ml/minute or less and can affect any system in the body. These include: weakness and fatigue, nausea, nocturia, pruritus, muscular twitches, cramps and pains. As the condition worsens the symptoms progress to include oedema (swelling of the face, limbs and abdomen), oliguria (greatly reduced volume of urine), dyspnoea (breathlessness), vomiting, confusion, seizures (Scott, 2013).

Chronic kidney disease may also be identified when it leads to one of its recognized complications, such as cardiovascular disease, anaemia, bleeding which is caused by impairment of platelet function, metabolic bone disease (known as Renal Osteodystrophy), myopathy, or pericarditis (NKF K/DOQI, 2002).

2.2.3 Laboratory Diagnosis

A variety of laboratory tests are available to assist clinicians in the assessment of kidney function and injury. Traditional markers of kidney function include testing urine for proteinuria as well as blood test for creatinine and clearance (GFR) tests. However, these tests have a number of limitations. Orthostatic proteinuria, diet, menstruation, transient fever, hydration status, and physical activity are all factors that may affect the level of proteinuria (Michael and Sushrut, 2012). The assessment of proteinuria as a marker of CKD can be limited by the potential for misclassification of individuals because of variability of protein levels in the individual's urine over time and the extent to which conditions at that time may obscure the true level (Aminu*et al*, 2005).Furthermore, besides diabetic

nephropathy, little is known of the renal outcome of patients with isolated microalbuminuria. Measurement of serum creatinine is simple but the general view is that up to 50% of nephrons can be lost before significant elevation of serum creatinine occurs (Christenssonet *al*, 2004).

Several serum creatinine–based equations have been developed to estimate GFR, the most notable being the Cockcroft–Gault, Modification of Diet in Renal Disease (MDRD), and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations for adults and the Schwartz equation for children. Although these equations generally increase the reliability of estimating the GFR, they all have limitations. For example, the MDRD equation is known to underestimate the GFR, particularly at lower creatinine concentrations, whereas the Cockcroft–Gault and Schwartz equations have been shown to overestimate the GFR, especially at lower creatinine concentrations (Michael and Sushrut, 2012).

Newer markers such as β2-microglobulin, cystatin C, and β-trace protein (BTP), have been evaluated as potential markers of GFR. In general, these proteins are freely filtered by the glomerulus, reabsorbed and catabolized, but not secreted by the renal tubules. As a result, reductions in GFR are associated with increased plasma concentrations(Michael and Sushrut, 2012).

Cystatin C, a 120-amino acid, 13-kDa protein belonging to the cystatin super-family of competitive inhibitors of lysosomal cysteine proteases, has a constant production rate by all nucleated cells. It is freely filtered at the glomeruli, and has no systemic reabsorption or renal tubular secretion. In addition, its serum level is not significantly influenced by age, gender, or muscle mass. Hence, cystatin C is superior to creatinine as a marker of kidney functions (Newman, 2002; Moses *et al*, 2012). Cystatin C has been proposed by several

groups and a recent meta-analysis to be a more sensitive and specific marker of kidney function in both children and adults (Dharnidharka*et al,* 2002). However, a study by Darcy *et al* (2008) showed that serum cystatin C is significantly related to gender, age, race, uric acid, and blood urea nitrogen in united states (US) adolescents. Abiodun*etal* (2012) reported that serum cystatin C level of children with HIV infection was 1.01 ± 0.44 mg/L, significantly higher than the mean value in the control group; and cystatin C-based eGFR showed high prevalence of CKD among HIV-infected children. A similar study documented significantly higher serum cystatin C levels in HIV-infected children than controls but serum cystatin C level and estimated GFR were not significantly different between the HIV-infected children with advanced disease and those with milder disease (Esezobor *et al,* 2010). Frans*et al* (2003) reported that cystatin C shows a high correlation with GFR measured with the continuous infusion iothalamate method, and gives a good estimate of GFR, more accurate and precise than serum creatinine and estimated GFR by Cockcroft and Gault formula.

Cystatin C levels have been reported to be altered in patients with cancer,thyroid dysfunctionand glucocorticoid therapy in somebut not allsituations (Arend, 2002). Levels seem to be increased in HIV infection, which might or might not reflect actual renal dysfunction. The role of cystatin C to monitor GFR during pregnancy remains controversial (Strevens, 2002; Akbari*et al*, 2005)

2.2.4 Treatment of CKD

The adverse outcomes of CKD can be prevented or delayed through interventions in the earlier stages of the disease, which can be detected through laboratory testing such as measurement of serum creatinine, estimation of GFR, measurement of urinary albumin excretion, urine microscopy for cellular elements and casts, and by radiologic

investigations. The therapeutic interventions that have proven effective include strict blood glucose control in diabetes, strict blood pressure (BP) control, use of angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin II receptor blockers (ARBs) and protein restriction **(**Alebiosu and Ayodele, 2005). Patients with end-stage renal disease require renal replacement therapy in form of dialysis or kidney transplant.

2.2.5 Prevention

The preventive strategies to stem the tide of CKD should involve educating the population on how to prevent renal disease; identifying those at risk of developing CKD; raising the awareness of the general public, policy makers, and health care workers; modifying the lifestyle of susceptible individuals; detecting early stage of CKD; arresting or hindering the progression of disease; and creating facilities for global assistance (Olugbenga*et al*, 2010). The control of hypertension, dyslipidaemia, proteinuria and obesity, also avoiding low birth weight, smoking, and heavy metals such as lead, are intervention strategies that will retard or prevent progression of renal diseases (Alebiosu and Ayodele, 2005).Given sufficient commitment to implementation of existing knowledge and a concurrent, continued dedication to acquiring new information, much of the suffering from CKD can be expected to be reduced and eliminated **(**Alebiosu and Ayodele, 2005).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 BACKGROUND OF STUDY AREA

This is a cross-sectional descriptive study, conducted at Ahmadu Bello University Teaching Hospital Shika, Zaria. The predominant occupation of the people of Zaria is farming but civil servants and businessmen also form a sizable part of the population. The predominant religions are Islam and Christianity. Zaria has a tropical climate with a temperature range from 15.3^0C to 40.2^0C sometimes. Zaria has two main seasons. The dry season from November to March while the rainy season lasts from May to October with long-term annual rainfall of 1040mm in about 90 rain days (ABU, 2014).

3.2 STUDY POPULATION (SUBJECTS)

For the purpose of this study, one hundred and fourteen (114) adult patients with chronic kidney disease attending Nephrology Clinic in ABUTH Shika, Zaria and and one hundred and fourteen (114) apparently healthy age and sex matched (controls) group were recruited over a period of four months in the year 2014. The control group was recruited from the apparently healthy adult population of surrounding settlements of Milgoma, Shika, Samaru and Zaria City.

3.2.1 Inclusion criteria

(i) Patients with newly diagnosed CKD, or on follow up, attending Nephrology Clinic at ABUTH Shika, Zaria who consent to participate in the study.

(ii) Similar number of apparently healthy subjects (controls) who consent to participate in the study.

3.2.2 Exclusion criteria

(i) Patients with cancer, thyroid dysfunction or on glucocorticoids therapy; from history and findings on physical examination.

(ii) Pregnant patients.

(iii) Control subjects with proteinuria.

(iv) Subjects who refuse to give consent.

3.2.3 Consent

Fully informed signed consent was obtained from each patient and control in the study.

3.2.4 Sample size

The sample size (n) was determined using the formula for the calculation of minimum sample size (Oyejide, 1992; Singha, 1996);

Estimated sample size is calculated using the formula below:

$n=z^2pq/d^2$

Where:

n=sample size, z=confidence interval (1.96), p=prevalence in target population (8%), q=1-p, d=precision (0.05)

$n= (1.96)^2\ 0.080 \times 0.920/ (0.05)^2 =113.1$

One hundred and fourteen (114) patients were recruited for the study. One hundred and fourteen (114) apparently healthy age-sex matched subjects were recruited as controls.

3.2.5 Ethical Approval

Ethical approval was obtained from the Ethics and Scientific Committee of ABUTH Zaria before embarking on the study.

3.3 STUDY PROTOCOL

Two separate questionnaires (appendix iii and iv) were administered to patients and controls respectively. These contain the biodata, history, physical examination and laboratory results of the participants.

The weight (kg) of all participants, wearing light clothing, was recorded to the nearest 0.5kg using Hanson standard bathroom (standing) weighing scale. Blood pressure was taken on the dominant arm using Accusons sphygmomanometer with an appropriate standard cuff of 12-13cm by 55cm size.

Serum cystatin C and creatinine assay was carried out. Urine microalbumin test was done on the control subjects to exclude those with microalbuminuria. Estimated GFR (eGFR) was calculated using the Cockcroft and Gault formula (Cockcroft and Gault, 1976)**:**

eGFR = (140 – Age (yr) x Wt (kg) x 1.2 / serum Cr (µmol/l) for male

= (140 – Age (yr) x Wt (kg) x 1.2 x 0.85 / serum Cr (µmol/l) for female

3.4 SPECIMEN COLLECTION AND PROCESSING

3.4.1 Blood Collection

Blood specimen was collected from a peripheral vein via antecubital venipuncture. The antecubital fossa was cleaned with methylated spirit and allowed to dry. A tourniquet was applied a few centimeters above the antecubital fossa to distend the veins. Five (5) ml of blood sample was then collected using a sterile 5 ml syringe and 21G needle. This was dispensed into a plain bottle and allowed to stand for about 30 minutes to clot. The clotted blood sample was then centrifuged for 10 minutes at 1000 rpm. The serum was separated from the cells and transferred into Bijou (sample) bottle and then frozen at -20^{o}C in deep freezer until the time for analysis within thirty days of collection.

3.4.2 Urine Collection

Urine sample bottle was provided to the control participants to produce about 10-15ml of random urine for microalbumin analysis. Urine sample from female controls was collected at least 3 days before or after their menstrual period to avoid false positive results. All subjects with proteinuria were excluded from the study.

3.5 CHEMICALS

The kits used for the measurement of serum creatinine was procured from Chemelex S.A. (08420 Canovelles-Bercelona) and that for cystatin C and Microalbumin from Beijing Share-Sun OET Co Ltd (China). All the chemicals and kits were of analytical grade or higher.

3.6 EQUIPMENT

Hettich Universal 32 Centrifuge (Germany) was used to spin the blood specimens. MRS V-6 General purpose UV/Vis Spectrophotometer (Israel) was used for the measurement of absorbance of creatinine while ATSA 01 Nephelometer (Hong Kong) was used for cystatin C and microalbumin assays.

3.7 ANALYTICAL METHODS

3.7.1 Creatinine (Jaffé, 1886)

3.7.1.1 Principle

Creatinine reacts with picric acid in alkaline medium forming a yellow orange color complex which is measured at 492nm:

Creatinine + Picric Acid $\longrightarrow$ Creatinine Picrate

3.7.1.2 Procedure

i) All the Reagents and samples were brought to room temperature.

ii) A working reagent was prepared by Mixing 1 volume of Reagent R1 with 1 volume of Reagent R2.

iii) Into each of test tubes marked Blank, Test and Standard 1.0 millilitre (mL) of the working reagent was added. One hundred (100) microlitre (µL) of sample and standard were added to corresponding tubes and mixed well.

iv) The initial absorbance A1 was read exactly after 30 seconds of mixing and the final absorbance A2 after 90 seconds at room temperature.

3.7.1.3 Calculation

Creatinine concentration in the sample was calculated using the following formula:

$$\text{Creatinine } (\mu\text{mol}/\text{L}) = \Delta A_{\text{test}} / \Delta A_{\text{standard}} \times 2(\text{Standard Concentration})$$

where, ΔA = change in absorbance(A2 – A1)

The values obtained were in mg/dL and thus converted to µmol/L using the conversion factor 88.4 as follows:

mg/dL X 88.4=µmol/L

3.7.2 Cystatin C (Kapmeyerand Sieber, 1981)

A latex immunoturbidimetric assay was used for the quantitative measurement of serum cystatin C, using kits manufactured by Beijing Share-Sun OET Co Ltd.

3.7.2.1 Principle

This Cystatin C test is based upon the reactions between Cystatin C and latex-covalently bound antibodies against human Cystatin C. The turbidity caused by these immune complexes is proportional to the cystatin C concentration in the sample and may be spectrophotometrically measured.

Cystatin C values are determined turbidimetrically using fixed time measurement with sample blank correction. The relationship between absorbance and concentration permits a multipoint calibration with a measuring range between 0 and 10 mg/L. The measuring temperature is 37ºC. The assay can be performed on all instruments allowing turbidimetric measurements at 500 to 600 nm.

3.7.2.2 Procedure

i) All reagents were brought to assay temperature (37^{o}C) by incubating for 3-5 minutes in the reagent incubator prior to use.

ii) The R1 cuvettes were unsealed and placed in the holder on the Nephelometer.

iii) Using a 10µL micropipette 5µL of serum was added into R1. Automatic mixing and 9 seconds countdown was started.

iv) Using a 200µL micropipette 55µL of R2 was then added. Automatic mixing and 9 seconds countdown was started.

v) The test countdown of 60 seconds was started: at the end the results was displayed on the screen of the Nephelometer and printed out.

3.7.3 UrineMicrolbumin Test.

The urine samples from the control participants were tested for albumin by immunoturbidimetric method.

3.7.3.1 Principle

The test is based upon the reactions between albumin in the urine sample and latex-covalently bound antibodies against human albumin. The turbidity caused by these immune complexes is proportional to the albumin concentration in the urine sample and may be spectrophotometrically measured.

Albumin values are determined turbidimetrically using fixed time measurement with sample blank correction. The relationship between absorbance and concentration permits a multipoint calibration with a measuring range between 0 and 10 mg/L. The measuring temperature is 37ºC. The assay can be performed on all instruments allowing turbidimetric measurements at 500 to 600 nm.

3.7.3.2 Procedure

i) All reagents were brought to assay temperature (37^{o} C) by incubating for 3-5 minutes in the reagent incubator prior to use.

ii) The R1 cuvettes were unsealed and placed in the holder on the Nephelometer.

iii) Using a 10µL micropipette 5µL of the urine sample was added into R1. Automatic mixing and 9 seconds countdown was started.

iv) Using a 200µL micropipette 55µL of R2 was then added after the 9 seconds countdown. Automatic mixing and 9 seconds countdown was started.

v) The test countdown of 145 seconds started: at the end the results was displayed on the screen of theNephelometer and printed out.

3.8 QUALITY CONTROL

Adequate quality control was observed when carrying out the analysis of the samples to ensure that the results obtained are reliable. This was done by analysing the samples in a batch together with quality control sera for specific analytes.

3.9 STATISTICAL ANALYSIS

The data were analysed using statistical package for the social sciences (SPSS) computer programme for windows (IBM Inc, USA) version 21. The mean values, range and standard deviation (SD) were calculated. Serum levels of cystatin C, creatinine and estimated GFR obtained from the patients were compared with those of the controls using the two tailed student's t-test. Correlation of serum cystatin C and creatinine with estimated GFR was carried out using Pearson's linear correlation analysis. A p-value of equal to or less than 0.05 ($p \leq 0.05$) was considered as statistically significant.

CHAPTER FOUR

4.0 RESULTS

4.1 DESCRIPTION OF THE STUDY POPULATION

One hundred and fourteen (114) patients with chronic kidney disease (CKD) were recruited for the study. These comprise of 59 male and 55 female patients, aged 18 to 85 years. Equal number of apparently healthy sex and age matched participants were recruited as controls. The mean age of the patients was 47.5 ± 15.3 years while that of the controls was 48.6 ± 13.4 years . The difference was not statistically significant with a p value of 0.559. Similarly, there was no statistically significant difference in sex, ethnicity and educational status between the patients and the controls with p values of 1.000, 0.291 and 0.422 respectively as shown in table 4.1.

Table 4.2 shows the mean (± SD) of body mass index (BMI), systolic (SBP) and diastolic blood pressure (DBP) of the patients and that of the controls. The mean BMI of patients with CKD was 25.78 ± 6.06 kg/m^2 while that of the controls was 21.12 ± 4.32 kg/m^2. The difference was statistically significant with p value of <0.0001. Similarly, the mean systolic and diastolic blood pressure (146.30 ± 25.91 mmHg and 92.55 ± 16.48 mmHg respectively) of the patients with CKD was found to be significantly higher than that (120.70 ± 9.79 mmHg and 79.34 ± 8.50 mmHg respectively) of the controls ($p<0.0001$).

Table 4.1: Demographic characteristics (mean±SD)of patients with CKD and apparently healthy controls.

Participants	n	Age (Years)	Sex	Ethnicity	Education
Patients	**114**	**47.50 ± 15.30**	**1.48 ± 0.50**	**1.73 ± 1.15**	**2.51 ± 1.08**
Controls	**114**	**48.60 ± 13.40**	**1.48 ± 0.50**	**1.57 ± 1.10**	**2.40 ± 1.21**
p-Value		**0.559**	**1.000**	**0.291**	**0.422**

n=sample size; SD= standard deviation

Table 4.2: Clinical characteristics (mean±SD)of patients with CKD and apparently healthy controls.

Participants	n	BMI ($kg/m^{2)}$	SBP(mmHg)	DBP(mmHg)
Patients	**114**	**25.78 ± 6.06**	**146.30 ± 25.91**	**92.55 ± 16.48**
Controls	**114**	**21.12 ± 4.32**	**120.70 ± 9.79**	**79.34 ± 8.50**
p-Value		**<0.0001**	**<0.0001**	**<0.0001**

n=sample size; BMI=Body mass index; SBP=systolic blood pressure; SDP=diastolic blood pressure; SD= standard deviation

4.2 BIOCHEMICAL CHARACTERISTICS OF THE STUDY POPULATION.

Serum cystatin C concentration of the control subjects ranged from 0.403 to 1.128 mg/L with a mean of 0.764 ± 0.215 mg/L. The reference interval was established as 0.297-1.153 mg/L using the formula of mean ± 2 standard deviations. Serum creatinine concentration ranged from 33 to 140 µmol/L with mean of 89.18± 24.99 µmol/L. The reference interval was therefore established as 39-139 µmol/L using the formula of mean ± 2 standard deviation. The estimated GFR ranged from 38-171 mL/Min/1.73m^2, with a mean of 84.58 ± 28.28 mL/min/1.73m^2, as shown in tables 4.3 and 4.4.

Serum cystatin C concentration of the patients ranged from 0.574 to 7.500 mgl/L with a mean of 3.106 ± 2.022 mg/L. Serum creatinine ranged from 47 to 2273 µmol/L with a mean of 610.83 ± 618.50 µmol/L, while the estimated GFR ranged from 3-163 mL/min/1.73m^2, with a mean of 34.41 ± 34.08 mL/min/1.73m^2.

Serum cystatin C and creatinine were higher while estimated GFR was lower in patients compared to that of controls. These differences between patients and controls in all the three parameters were statistically significant with p value <0.0001 as demonstrated in table 4.3.

Table 4.3: Serum Cystatin C, creatinine andeGFR(Mean±SD) of patients and apparently healthy controls.

Participants	N	Cystatin C (mg/L)	Creatinine (µmol/L)	eGFR(mL/min/1.73M^2)
Patients	114	3.106 ± 2.022	610.83 ± 618.50	34.41 ± 34.08
Controls	114	0.764 ± 0.215	89.18 ± 24.99	84.58 ± 28.28
p-Value		<0.0001	<0.0001	<0.0001

n=sample size; eGFR= estimated Glomerular Filtration Rate; SD= standard deviation

Table 4.4: Reference intervals for serum cystatin C and creatinine.

Analyte	Mean	SD	Reference intervals (Mean ± 2SD)
Cystatin C (mg/L)	0.764	0.215	0.297 – 1.153
Creatinine (µmol/L)	89.18	24.99	39 – 139

SD= standard deviation

4.3STAGING OF THE CKD PATIENTS USING THE eGFR

Table 4.5 shows the distribution of the patients by stage of chronic kidney disease (CKD). Only 24 patients (21%) hadeGFR of 60 mL/min/1.73m^2 and above; that is stage 1 and 2 (the early stages of CKD). Twenty six (26) patients had eGFRof 30-59 mL/min/1.73m^2 while fifteen (15) had eGFR of 15-29 mL/min/1.73m^2 ; that is stage 3 and stage 4 CKD, respectively. The remaining 49 (43%) of the patients had eGFR less than 15 mL/min/1.73m2, that is the terminal or stage 5 CKD. This shows that majority of the patients are in advanced stages (stage 3-5) of the disease.

Table 4.5: Distribution of Patients by stages of CKD usingeGFR (mL/min/1.73m^2)

Stage by eGFR (mL/min/1.73m^2)	Number of Patients	Percentage
Stage 1 (>90)	8	7.0
Stage 2 (60-89)	16	14.0
Stage 3 (30-59)	26	22.8
Stage 4 (15-29)	15	13.2
Stage 5 (<15)	49	43.0
Total	114	100.0

CKD=chronic kidney disease; eGFR= estimated Glomerular Filtration Rate

4.4THE BIOCHEMICAL ANALYTES AND STAGES OF CHRONIC KIDNEY DISEASE.

The results for the serum levels of cystatin C and creatinine, differentiated for the 5 stages of CKD, are given in table 4.6. The mean serum cystatin C levels were closer to the upper reference limit in stage 1 and exceeded it in stage 2 CKD. On the other hand, the mean serum creatinine levels in stage 1 and stage 2 were within the normal reference intervals generated from the control subjects and only exceeded the upper reference limit at stage 3 CKD

Table 4.6: Serum levels of cystatin C and creatinine (Mean ± SD)of patients at the different stages of CKD.

Stages	n	Creatinine (µmol/L)	Cystatin C (mg/L)
Stage 1	**8**	**70.75 ± 22.75**	**1.032 ± 0.401**
Stage 2	**16**	**105.13 ± 25.48**	**1.357 ± 0.839**
Stage 3	**26**	**140.98 ± 36.81**	**1.655 ± 0.718**
Stage 4	**15**	**305.17 ± 118.98**	**2.584 ± 1.126**
Stage 5	**49**	**1207.00 ± 499.99**	**4.945 ± 1.527**
All stages	**114**	**610.83 ± 618.50**	**3.106 ± 2.022**

CKD=chronic kidney disease n=sample size; eGFR= estimated Glomerular Filtration Rate

4.5 RELATIONSHIP BETWEEN SERUM CYSTATIN C, CREATININE AND eGFR AMONG THE CKD PATIENTS.

The relationship of serum cystatin C, creatinine and eGFR among the CKD patients is shown in table 4.7 and illustrated in figures 4.1, 4.2 and 4.3.

There was a strong positive correlation between serum cystatin C and creatinine in the patients ($r = 0.811$; $p < 0.0001$). The negative correlation between cystatin C and the estimated GFR ($r = -0.708$; $P < 0.0001$) was stronger than that between creatinine and the estimated GFR ($r = -0.694$; $P < 0.0001$).

Table 4.7: Relationship between serum cystatin C, creatinine andeGFR among the CKD patients

	Cystatin C : Creatinine	Cystatin C:eGFR	Creatinine :eGFR
Pearson correlation	**0.811**	**-0.708**	**-0.694**
p-value	**<0.0001**	**<0.0001**	**<0.0001**

CKD=chronic kidney disease; eGFR= estimated Glomerular Filtration Rate

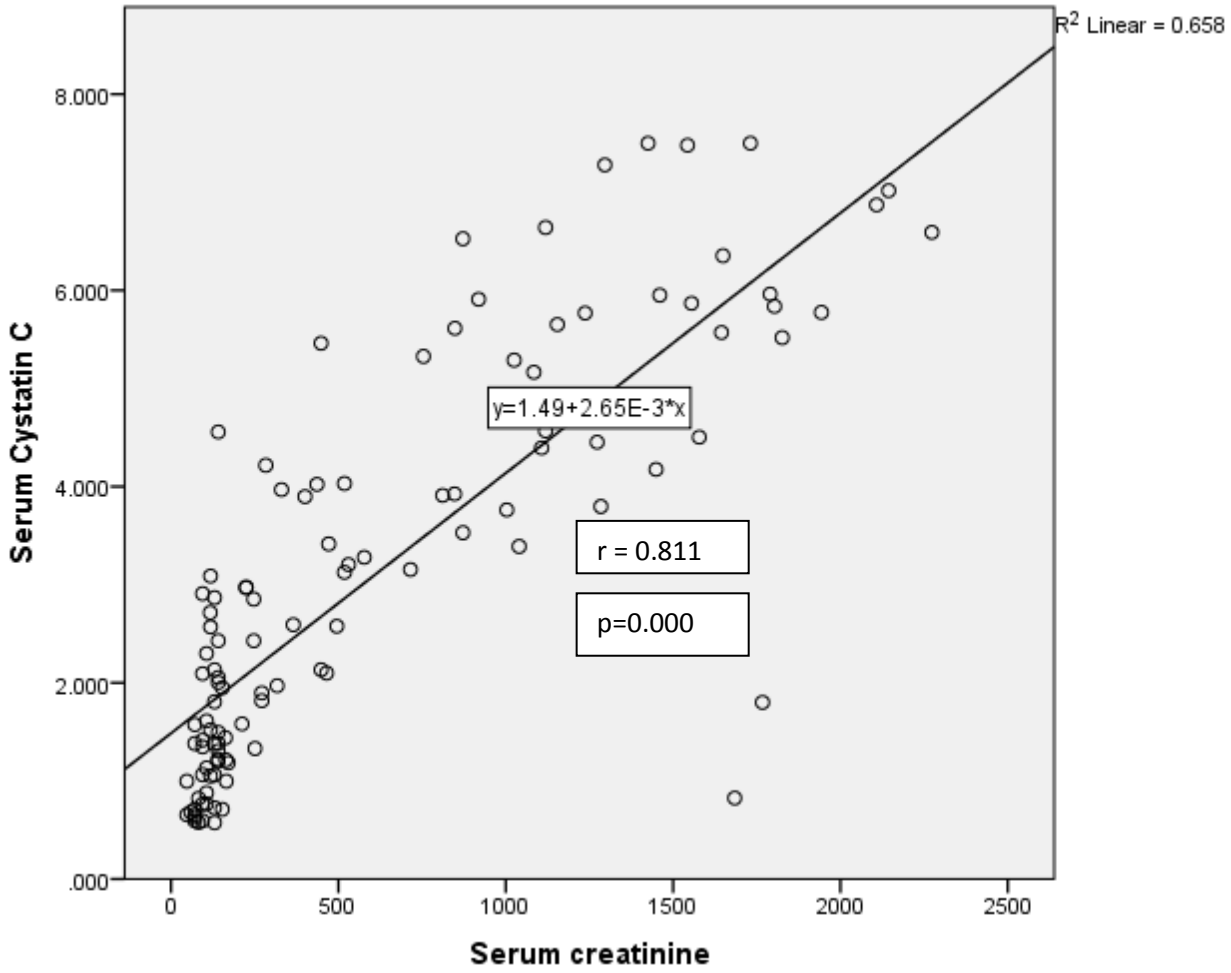

Figure 4.1: Relationship between serum cystatin C and creatinine

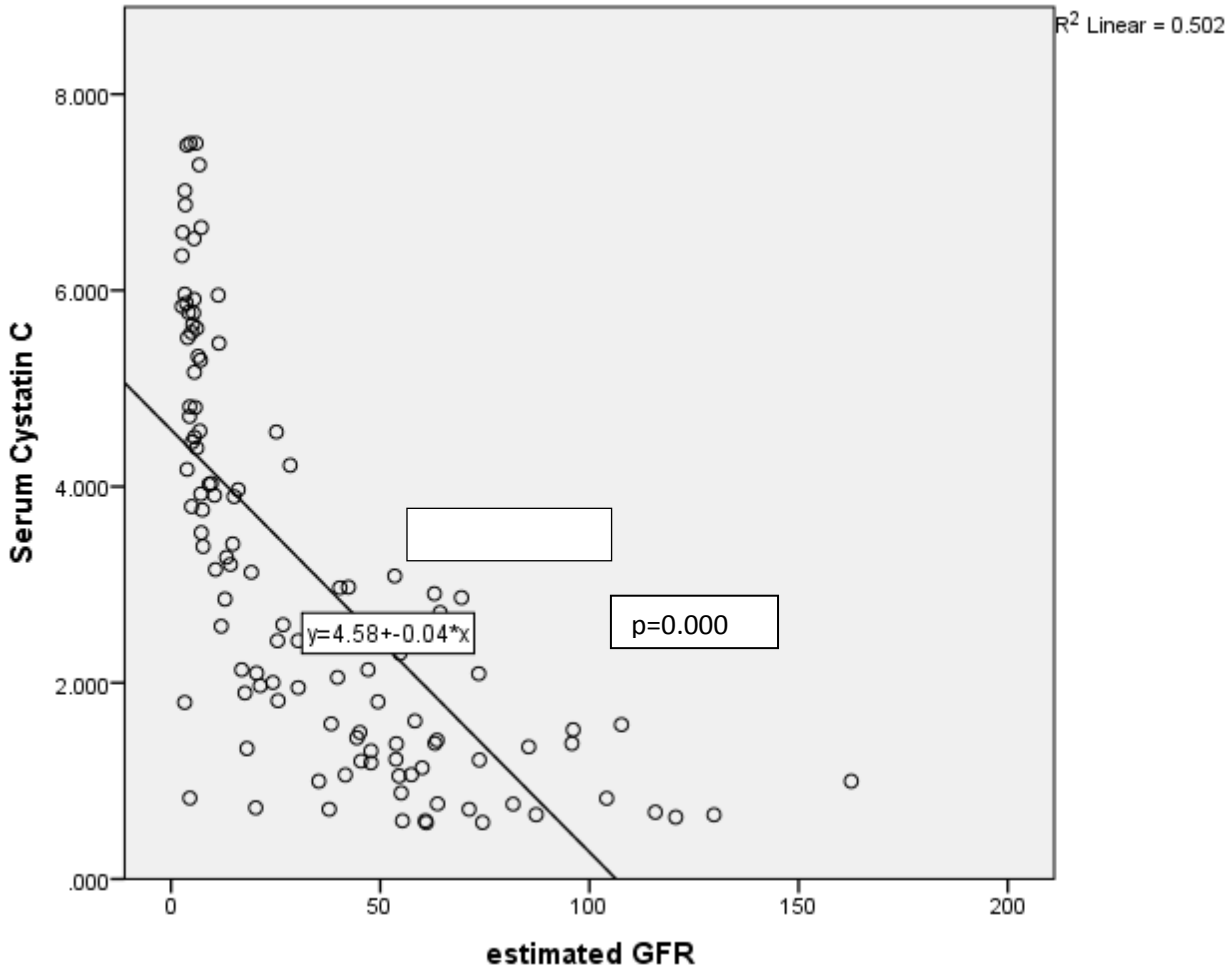

Figure 4.2: Relationship between serum cystatin C and the estimated GFR

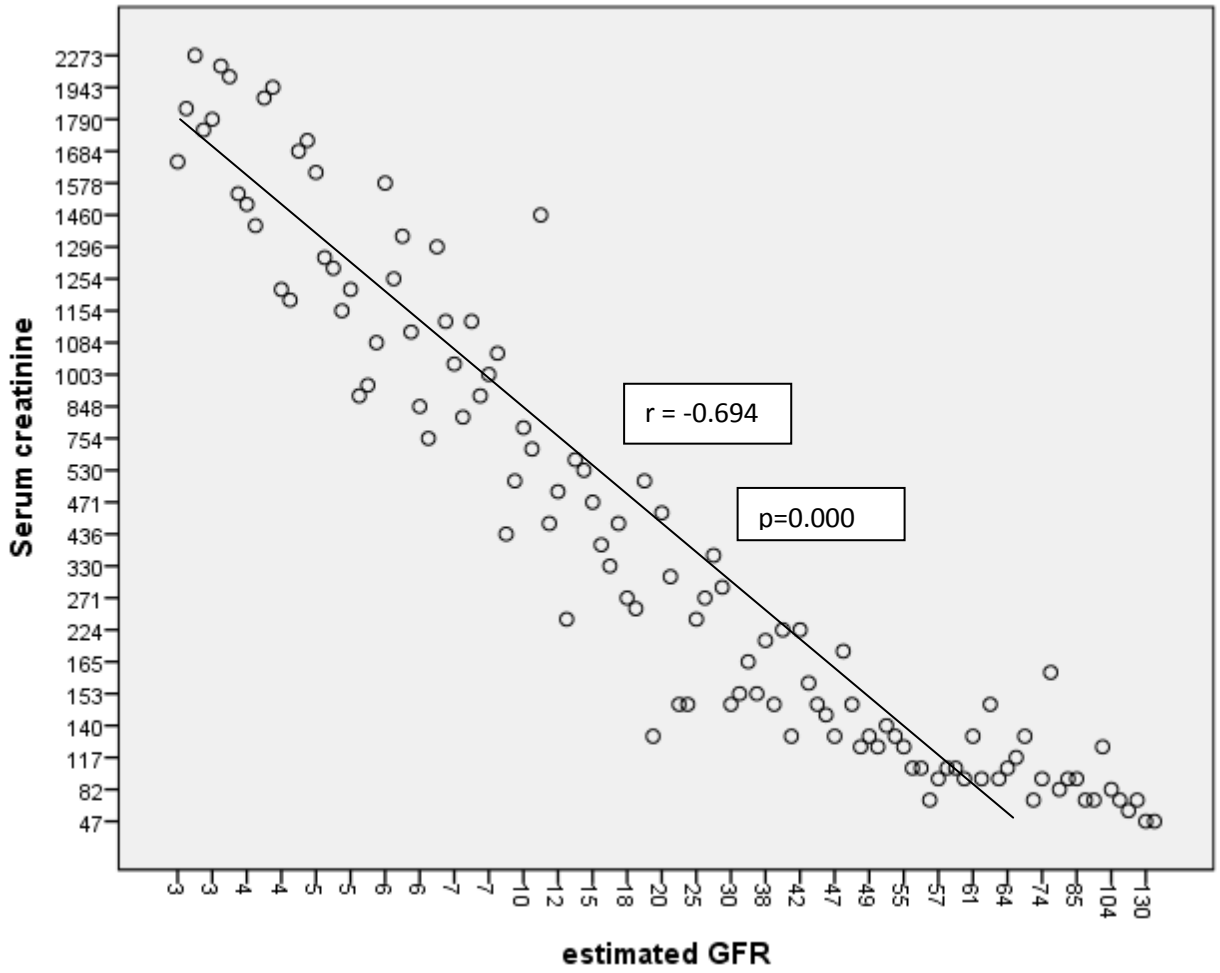

Figure 4.3: Relationship between serum creatinine and the estimated GFR

CHAPTER FIVE

5.1 DISCUSSION

The limitations of serum/plasma creatinine as a measure of glomerular filtration rate (GFR) have led to an extensive search for a more sensitive laboratory marker of impaired renal function (Westhuyzen, 2006).

The upper limits of the reference intervals for cystatin C and creatinine obtained in this study were slightly higher than that obtained by Finney et al (2000) on 309 healthy blood donors. In their study, the 95% reference intervals for cystatin C and creatinine, regardless of gender, were 0·51-0·98 mg/L and 68-118 µmol/L, respectively.Similarly, in a study byUhlmann et al (2001) on 139 healthy volunteers , using similar methods to the ones used in this study, the values obtained were comparable to, although approximately 10% lower. The higher upper limit of the reference interval in this study may be due to the difference in method used for the calculation of the reference intervals. Differences in standardization between methods also result in different reference ranges and different test outcomes (Dharnidharka*et al*, 2002).

The mean serum cystatin C and creatinine of the patients were significantly higher while estimated GFR significantly lower than that obtained by Hojs*et al* (2006) in their study on serum cystatin C as an endogenous marker of renal function in patients with mild to moderate impairment of kidney function. Similarly, a study by Rigalleau*et al* (2008) on 124adult patients with CKD reported lower mean cystatin C and creatinine and higher eGFR than that obtained in this study. This is expected because most of the patients in the present study were in advanced stages of CKD. Cystatin C is freely filtered by the glomeruli, reabsorbed and degraded by tubular cells. Glomerular dysfunction in CKD leads to retention of cystatin C in the blood.

The results for the serum markers for GFR for the different stages of CKD showed that the mean serum cystatin C concentration was closer to the upper reference limit at stage 1 and became abnormal at stage 2 CKD. On the other hand, the mean serum creatinine concentration did not exceed the upper limit of its reference interval until stage 3 CKD. These findings are similar to those by Frans et al (2003), and thereby proves the superiority of cystatin C in early stages of chronic kidney disease. Macisaac*et al* (2007) reported that serum cystatin C level cut-off > 1.10 mg/L had the best test characteristics as a screening tool for detecting moderate CKD (< 60 ml/min/1.73 m^2) when compared with creatinine-based methods. Similarly,the diagnostic accuracy of serum cystatin C was better than serum creatinine for stage 1 and 2 chronic kidney disease (CG-CCr cut-off value of 90 ml/min and 60 ml/min) in a study on Taiwanese with type 2 diabetes mellitus (Yang *et al*, 2007). Unlike creatinine, cystatin C is not secreted by renal tubular and gastrointestinal cells.

There was a strong correlation between serum cystatin C and creatinine in the patients. This finding agrees closely with that among 110 patients in the study by Uhlmann*et al* (2001) mentioned earlier. In the study of Le Bricon*et al* (2005) on 26 ICU patients, a slightly stronger correlation between Cystatin C and creatinine was demonstrated. The strong correlation between serum cystatin C and creatinine suggests that in addition to its superiority in detecting early CKD, cystatin C is also useful in monitoring the patients.

The correlation between serum cystatin C and the eGFR in the patients was also significantly stronger than that between creatinine and eGFR. Many studies have reported similar findings. In the studies by Frans*et al* (2003) and Cepeda*et al* (2007) the correlation between cystatin C and GFR was highly significant and significantly better than between creatinine and GFR. These findings are slightly better than those in the present study probably because they used [^{125}I] iothalamate clearance to measure GFR. Similarly, the

correlation of cystatin C and creatinine with GFR in the study by Pucci*et al* (2007) was higher. They measured serum creatinine by the Jaffe's method and cystatin C by the immunoturbidimetric method, on 288 diabetic patients with renal impairment, but used iohexol clearance to measure GFR.

Several other studies have suggested that cystatin C is more sensitive to small changes in GFR than serum creatinine (Finney *et al*, 2000;Buysschaert*et al*, 2003; Stefan*et al*, 2007). In a large study in US it was demonstrated that adding cystatin C to the combination of creatinine and ACR (urine albumin-to-creatinine ratio) measures improved the predictive accuracy for all-cause mortality and end-stage renal disease (Peralta*et al*, 2011).

CHAPTER SIX

6.0 SUMMARY, CONCLUTION AND RECOMMENDATIONS.

6.1 SUMMARY

The findings from this study include the following;

a. Serum cystatin C and creatinine were significantly higher while estimated GFR was significantly lower in patients than the control subjects.
b. Serum cystatin C concentration was elevated earlier than that of serum creatinine in patients with CKD.
c. There was strong positive correlation between serum cystatin C and creatinine in patients with CKD.
d. The negative correlation between serum cystatin C and the estimated GFR was stronger than that between serum creatinine and the eGFR.

6.2 CONCLUSION

In conclusion, the findings from this study support the use of cystatin C as a better endogenous marker for the assessment of CKD than serum creatinine. Serum cystatin C concentration was elevated earlier in CKD when serum creatinine was still within its reference intervals and also showed a better correlation with the eGFR. This will permit early detection and better management of patients with CKD.

6.3 RECOMMENDATIONS

It is recommended from the findings of this study that:

1. Serum cystatin C may replace serum creatinine and eGFR as a routine test in the evaluation of renal function in patients with chronic kidney disease.
2. Large studies be carried out in patients at risk of kidney disease such as the hypertensive and diabetic patients to assess the value of this important marker in early detection of mild renal impairement.

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APPENDIX I

PARTICIPANT INFORMATION FORM

Serial No. ……..

Hospital No. …….

Age …….

Information form to participate in a study.

My name is RabiuAdamu, a lecturer and MSc student in the Department of Chemical Pathology.

1. This study is **MEASUREMENT OF SERUM CYSTATIN C, CREATININE AND ESTIMATED CREATININE CLEARANCE IN CHRONIC RENAL DISEASE PATIENTS IN ABUTH, ZARIA**. The procedure involves blood and urine collection and tests.
2. The tests will be of no cost to you.
3. The result from the study will be treated with strict confidentiality.
4. You are free to participate or decline in this study without any consequence.
5. The study will hopefully improve your present health status and that of others in the future.

If you desire to participate in this study kindly sign the attached form.

Thank you.

RabiuAdamu

Phone contact: 08035970111

Email address: mrajano@yahoo.com

APPENDIX II

INFORMED WRITTEN CONSENT

I …….. of ……(address) agree to participate in the study: **Serum cystatin C, creatinine and estimated creatinine clearance in chronic renal disease patients in ABUTH, Zaria**. The full procedure before and after the test have been explained to me.

I understand that a sample of my blood and urine will be taken for tests and that if I so wish the results will be communicated to me in confidence and at no extra cost to me. I understand that I can opt out at any stage without been denied treatment.

I make this consent wilfully without being subjected to any pressure.

Participant's Name…… Signature……

Witness's Name……. Signature……

Researcher's Name…… Signature…….

APPENDIX III

PATIENTS QUESTIONAIRE

Personal Data

Name:…….

Serial No: …….

Hospital No: ……

Age (years) …… Gender……

Nationality…….Ethnicity……..

Occupation……

Address:…….

Education: primary 1 secondary 2 tertiary 3 None 4

Weight (Kg) ……. Height (m) ……BMI (kg/m^2)……..

Systolic BP (mm Hg) ……..Diastolic BP (mm Hg)

Diagnoses: …….

Duration of disease or condition (years)……

Treatment modality (conservative, dialysis, kidney transplant)

Medications (Steroids, diuretics, analgesics)…….....

Other medical conditions……

LABORATORY INVESTIGATIONS

RESULTS

Creatinine (µmol/L)……... eCrCl (ml/min/1.73m^2)……

Cystatin C (mg/L)……. ….

eCrcl=estimated creatinine clearance

APPENDIX IV

CONTROLS QUESTIONAIRE

Personal Data

Name:…….

Serial No: …….

Hospital No: ……

Age (years) …… Gender……

Nationality…….Ethnicity……..

Occupation……

Address:…….

Education: primary 1 secondary 2 tertiary 3 None 4

Weight (Kg) ……. Height (m) ……BMI (kg/m^2)……..

Systolic BP (mm Hg) ……..Diastolic BP (mm Hg)……..

Any underlying medical conditions……

i) Thyroid disease

ii) Cancer

iii) Others……

Any medications (Steroids, diuretics, analgesics)…….....

LABORATORY INVESTIGATIONS

RESULTS

Creatinine (µmol/L)……... eCrCl (ml/min/1.73m^2)……

Cystatin C (mg/L)…….

Urine albumin (mg/L)……..

eCrcl=estimated creatinine clearance

APPENDIX V

HEALTH RESEARCH ETHICS COMMITTEE

AHMADU BELLO UNIVERSITY TEACHING HOSPITAL

SHIKA - ZARIA, NIGERIA.

E-mail: abuth@yahoo.com *Website: www.abuth.org*

Chairman of Board: Chief. Shuaib Oyedokun Afolabi *FnII*
Chief Medical Director: Prof. Lawal Khalid, *MBBS, FMCS, FWACS, FRCS(ED) mnI*
Chairman, Medical Advisory Committee: Prof. Abdullahi Mohammed, *MBBS, FWACP, FICS*
Director of Administration: Barr. Ishak Bello, *LL.B, BL., LL.M, PGDM, AHAN, FCAI*

Our Ref: ABUTH/HREC/TRG /36

Your Ref: _____

Date: 23rd July, 2014

ABUTH HREC FULL ETHICAL CLEARANCE CERTIFICATE

Evaluation of Serum Cystatin C, Creatinine and Estimated GFR, in Patients with Chronic Kidney Disease in Zaria, Nigeria.

ABUTH Ethics Committee assigned number:	-	**ABUTH/HREC/K58/2013**
Name of the principal Investigator:	-	**Dr. Rabiu Adamu,**
Address of the Principal Investigator:	-	**Department of Chemical Pathology ABUTH-Zaria**
Date of receipt of valid application:	-	**14th April, 2014**
Date of meeting when final determination on ethical approval was made:	-	**20th & 21st May, 2014**

This is to inform you that the research described in the submitted protocol, the consent forms and other participant information materials have been reviewed and ***given full approval by the Health Research Ethics Committee.***

Please note: this approval dates from **24th July, 2014– 24th July, 2015**.

No participant recruitment into this research may be conducted outside these dates.

All informed consent forms in this study must carry the ABUTH HREC number assigned to this research and the duration of ABUTH HREC approval of the study.

This HREC expects that you submit your application as well as an annual report for ethical clearance renewal 3 months prior to expiration of study dates. This is to enable you obtain renewal of your approval and avoid interruption of your research.

If there is delay in starting the research, please inform the ABUTH HREC so that starting dates can be adjusted accordingly.

No changes are permitted in the research without prior approval by ABUTH HREC, except in circumstances outlined in national code for Health Research Ethics: http://www.nhrec.net.

ABUTH HREC reserves the right to conduct compliance assessment visits to your research site without prior notification.

Prof. A. I. Mamman
Chairman, ABUTH HREC