CYSTATIN C AND BETA-2-MICROGLOBULIN IN DIABETIC AND HYPERTENSIVE NEPHROPATHY AND THEIR EFFECT ON CHRONIC KIDNEY DISEASE

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DECLARATION

The experimental work described in this thesis was carried out at the Department of Molecular Medicine, KNUST, the Regional Hospital, Koforidua, Kibi and Enyersi Government Hospitals in the Eastern Region. This work has not been submitted for any other degree

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ABSTRACT

Diabetic and hypertensive nephropathies are the major cause of chronic kidney disease and the number one cause of cardiovascular deaths worldwide. Glomerular filtration rate (GFR) is an important indicator of kidney function, essential for diagnosing, evaluating and managing chronic kidney disease (CKD). GFR can be estimated using creatinine and serum cystatin C. However, the estimation of GFR by using serum creatinine is documented to be affected by various factors such as age, sex and mass in the muscle and has led to the misclassification of CKD patients. The study therefore sought to evaluate the value and possible clinical applications of cystatin C and Beta 2 microglobulin as biomarkers for changes in GFR. The study adopted a cross-sectional design which involved 182 patients who had diabetes and or hypertension. The area under the curve (AUC) for Cystatin C for DM was 0.861, HTN was 0.731 and DM+HTN was 0. 758.The area under curve (AUC) for B2M for DM was 1.00, HTN was 0.8373 and DM+HTN was 0. 896.. The area under curve (AUC) for Cys C/B2M for DM was 0.765, HTN was 0.614 and DM+HTN was 0.757. There was a significant negative correlation (r= -0.947; p=0.004) between eGFR and age among participants with DM. Significant negative correlation was observed between eGFR and urea (r= -0.624; p=0.04), Total protein (r= -0.692; p=0.018), uric acid (r= -0.792; p=0.004) and Beta 2 Microglobulin (r= 0.743; p=0.009) among participants with HTN. There was a significant negative correlation between eGFR and age (r= -0.525; p=0.010), urea (r= -0.786; p<0.0001), uric acid (r= -0.464; p=0.026), cystatin C (r= -0.761; p<0.0001) and Beta 2 Microglobulin (r= -0.901; p<0.0001) among participants with both DM+HTN. Among diabetic (DM) participants without CKD, eGFR correlated negatively with age (r= -0.705; p=0.0002), and cystatin C (r= -0.587; p=0.003). Among hypertensives (HTN) participants without CKD, eGFR correlated negatively with age (r= -0.337; p=0.025), total protein (r= -0.361; p=0.016), cystatin C (r= -0.339; p=0.025) and Beta 2 Microglobulin (r= -0.429; p=0.004). eGFR negatively correlated with age (r= -0.505; p<0.0001), urea (r= -0.264; p=0.0214), uric acid (r= -0.264; p=0.021), cystatin C (r= -0.520; p= <0.0001) and Beta 2 Microglobulin (p= 0.500; p<0.0001). Serum Cystatin C and Beta 2 microglobulin have acceptable and significant sensitivities and therefore can be used as markers for renal impairment.

TABLE OF CONTENT

KNUST

KNUST

KNUST

LIST OF TABLES

KNUST

LIST OF FIGURES

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ABBREVIATIONS

ACR	-	Albumin to Creatinine Ratio
AUC	-	Area under the Curve
B2M	-	Beta 2 Microglobulin
BSA	-	Basal Surface Area
BUN	-	Blood Urea Nitrogen
Ccr	-	Creatinine Clearance
CG	-	Cockcroft- Gault
CHRPE	-	Committee on Human Research Publication Ethic
CI	-	Confidence Interval
CKD	-	Chronic Kidney Disease
CKD-EPI	-	Chronic Kidney Disease-Epidemiology Collaboration
CVD	-	Cardiovascular Disease
Cyc C	-	Cystatin C
DM	-	Diabetes Mellitus
DN	-	Diabetic Nephropathy
DTPA	-	Diethylenetriaminopentaacetic Acid eCcr
-	Estimated Creatinine Clearance EDTA	-
	Ethylenediaminetetraacetic Acid eGFR	-
	Estimated Glomerular Filtration Rate	
EQC	-	External Quality Checks
ESRD	-	End Stage Renal Disease
FGF	-	Fibroblast Growth Factor

GBM	-	Glomerular Basement Membrane
GFR	-	Glomerular Filtration Rate
IDMS	-	Isotope Dilution Mass Spectrometry
K/DOQI	-	Kidney Disease Outcomes Quality Initiative
KATH	-	Komfo Anokye Teaching Hospital
LDL-C	-	Low Density Lipoprotein Cholesterol
MDRD	-	Modification of Diet in Renal Disease
NCV	-	Nerve Conduction Velocity
Pcr	-	Plasma Concentration
PTH	-	Parathyroid Hormone
ROC	-	Receivers Operating Characteristics
Scr	-	Serum Creatinine
SPSS	-	Statistical Package for Social Scientist

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CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

Diabetic and hypertensive nephropathies are the major cause of chronic kidney disease and the number one cause of deaths from heart related diseases worldwide (Gress *et al.,* 2000). Fioretto *et al.* (2007) categorized diabetic nephropathy into two main stages namely, microalbuminuria and macroalbuminuria. According to them, the optimal health range of micro- and macro-albuminuria are based on random judgment such that patients in the high optimal range of albuminuria are vulnerable to progress to micro- or macroalbuminuria. The prevalence of diabetic nephropathy (DN) is different in relation to ethnic groupings such that it is more prevalent among AfroAmericans, people of Asianic origin and Native-Americans than in whites of European origin (Dronavalli *et al.,* 2008). Afro-Brazilians are more likely to progress to end-stage renal disease (ESRD) compared to natives of European countries, however the presence of micro- or macro-albuminuria are not different (Young *et al.,* 2003).

The main pathophysiological changes in DN in persons who have type 1 diabetes mellitus (DM) are noted by a thickened basement membrane of the glomerulus (GBM), an expanded mesangium, nodular sclerosis – Kimmelstiel-Wilson change, diffuse glomerular sclerosis, tubular interstitial fibrosis, arteriosclerosis and hyalinosis of kidney blood vessels (Hartog *et al.,* 2009). In addition, changes in the anatomy of the interstices occur, and also hyalinization of the afferent and efferent glomerular arterioles (Gerchman *et al.,* 2008).

According to Mogensen (1984), the outcome of albuminuria in damages to the kidney in type 1 and 2 diabetics was established around the 1980's and this led to the term initial nephropathy. This develops in about 20-30% of patients who have nephropathies (Viberti *et al.,* 1982). Kidney disease in the US is on the increase with over 3000 cases of Chronic Kidney Disease (CKD) reported yearly (National Kidney Foundation, 2012). According to the National Kidney Foundation (2002), chronic kidney disease (CKD) is characterized by a continuous decline in renal function spanning months or years and may also be defined as a creatinine- based estimated Glomerular Filtration Rate below 60mL/min/1.73m^2 or a urine albumin- to- creatinine ratio (ACR) of 30mg/g or above. The condition is characterized by a high risk of bad sequels such as mortality, heart related events, and the setting in of terminal kidney disease (Go *et al.,* 2004; Grubb *et al.,* 2005).

It is reported that people with heart diseases related to hypertension are highly at risk of developing type 2 diabetes, which is a relevant absolute risk determinant for CKD (Chobanian *et al.,* 2003; Fields *et al.,* 2004). Since the co-existence of hypertension and diabetes have a great effect on malaise and loss of lives, much focus has been based on ways to lower the co-existence of diabetes and hypertension. (Kannel *et al.,* 2000). In comparison to glomerular capillaries other capillaries are strongly porous to water and relatively not porous to sizable molecules (Stevens *et al.,* 2006). Gerchman *et al.* (2008) opined that, the extent of damage to the glomerulus is comparative to the value of GFR, span of diabetes mellitus, and control of blood sugar.

According to Viberti *et al*. (1982) and Dronavalli *et al.* (2008), the most suitable internal biomarker should have a steady production rate, steady ambient levels (not affected by changes in the pathology), not able to bind to proteins, must have the ability to be freely filtered by the kidneys and should not also be re-absorbed or secreted. To assess

glomerular function, some biomarkers Beta 2-microglobulin, urea, retinol-binding protein and creatinine have been used as internal biomarkers of GFR, by assaying for them in plasma. The most often used biomarker for assessing kidney function are creatinine clearance (CCr) and serum creatinine (USRDS, 2003). However, Stevens *et al.* (2007) reported that serum creatinine concentrations are impacted by muscle mass, age and sex. Therefore, estimating GFR based on serum creatinine alone cannot be relied on when determining the functions of the kidneys when GFR is above 60mL/min/1.73m^2 (Hartog *et al.,* 2009). This suggests that, current practices and staging systems that basically rely on serum creatinine may wrongly stratify persons when determining renal function.

1.2 PROBLEM STATEMENT

Glomerular filtration rate is an important tool of renal function, essential in the holistic approach to deal with CKD (Levey *et al.,* 2006). GFR can be estimated using creatinine and cystatin C. However, estimation of GFR using serum creatinine is documented to be affected by various determinants such as age, sex and mass in the muscle and has led to the misclassification of CKD patients (Stevens *et al.,* 2006). Unlike serum creatinine, rate of production, measure of cystatin C and Beta 2- microglobulin (B2M) are not impacted by muscle mass, gender or strain and have less or no evaluation limitations. Serum cystatin C and B2M biomarkers used in the detection of kidney disease are better predictors of mortality and heart related events than serum creatinine and are minimally impacted by age, sex or mass in the muscle
(Stevens *et al.,* 2006; Menon *et al.,* 2008).

Despite the potentials of cystatin C and Beta 2 microglobulin biomarkers, little evidence is available on their roles in renal impairments in Ghana. The correlation between

cystatin C levels, B2M, serum creatinine and estimated GFR in renal impairments is also unclear. A study that ascertains how the different markers could be used as risk factors for prognosis in CKD was, therefore, paramount. Hence, this study sought to determine the diagnostic sensitivity of cystatin C and B2M and their effect on CKD. The study was aimed at improving the accuracy in classification of CKD patients in Ghana and provide adequate baseline data for risk prediction and distinguish important prognosis in CKD.

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1.3 OBJECTIVES

The main purpose of the research work was to evaluate the diagnostic value and possible clinical applications of cystatin C and Beta 2 microglobulin biomarkers for changes in GFR.

The specific objectives of the study were to:

- Determine the diagnostic value of serum cystatin C as a measure for GFR.

 Determine the diagnostic value of Beta 2 microglobulin in kidney diseases

- Assess the effect of a combined marker approach in diagnosing CKD.

 Determine the correlation between cystatin C, B2M, creatinine and estimated GFR.

1.4 RESEARCH QUESTIONS

1. What is the sensitivity of serum cystatin C as a measure for GFR?

2. Does Beta 2 microglobulin has diagnostic value(s) in CKD
3. How accurate is the combined marker approach in diagnosing CKD patients?

4. Is there any correlation between cystatin C levels, serum creatinine, B2M and estimated GFR?

1.5 SIGNIFICANCE OF STUDY

The study evaluated the value and possible clinical applications of cystatin C and Beta 2 microglobulin biomarkers for changes in GFR. Contributions of the study to the existing body of knowledge in theory and practice cannot be underestimated. Findings from this study would provide insight on the sensitivity of other biomarkers such as Beta-2-microglobulin and Cystatin C. This would help prevent over-reliance on creatinine as the primary measure of GFR; thus indirectly preventing misclassification of chronic kidney disease.

Again, findings from this study would help produce correlations between cystatin C levels, B2M, serum creatinine and estimated GFR. This would give us an idea as to the degree to which these measures are associated with each other and whether their associations are significant or not. Knowledge of the extent of relationship between the said variables may allow for manipulation of one variable using the other; or, gaining insight about one variable whilst monitoring the other variable with which it pairs.

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CHAPTER 2

LITERATURE REVIEW

2.1 OVERVIEW AND DEFINITION OF TERMINOLOGIES

Chronic renal disease is a worldwide public health issue which affects 5–10% of people in the world (Lamb *et al.,* 2003; Coresh *et al.,* 2007). Even, in the developed countries such as United States, Chronic kidney disease (CKD) is seen as a serious health problem which affect about 13% of the populace (KDOQI, 2006). In recent decade, there has been an increasing evidence of renal failure with bad prognosis and huge financial burden in Ghana (Levey *et al.,* 2005). It has been found to account for 8-10% of medical admissions in Ghana (Plange - Rule *et al.,* 1999). In 2011, Osafo *et al.,* recorded a prevalence of 46.9% in hypertensives in a Ghanaian community. The number of patients presenting with CKD is on the rise because of the increasing number of people with diabetes and hypertension as well as the growing elderly population.

The condition is normally characterized by an absence of symptoms for a period, which can be detected clinically. Patients who have had early treatment have delayed their progress of CKD, reduced the setting in of complications such as heart related diseases. (Burke *et al.,* 2003). There is a significant inter-connection between CKD, diabetes and Hypertension and as people advance in age they develop the risk of having CKD.

Chronic kidney disease ranges from mild, to severe (D'Haese *et al.,* 2003). When CKD progress to the point of the kidneys failing, a patient needs a transplant of the kidneys or to be put on dialysis which is necessary to aid life and since the disease goes bad over time; prompt diagnosis and treatment usually reduces progression to advance stages. (Freemont *et al.,* 2005a).

Hypertension is defined as blood pressure that is too high (Atkins *et al.*, 2004). One function of the heart is to create pressure to force blood to various parts of the body. However, the narrowing of arteries in the heart create a high pressure than normal so as to pump blood to all parts of the body. Although, there is no cure for high blood pressure, when the risk determinants are known, it helps to manage and control the disease (D'Haese *et al.*, 2003).

The most well-known types of diabetes are the type 1 and type 2 (Sterrett *et al.*, 2007). In type 1 diabetes there is a low level of insulin due to functional or anatomical defect of the pancreas. This is usually seen in young adults. Insulin is required to treat type 1 diabetes (Fujii *et al.*, 2007).

Type 2 diabetes on the other hand, develops when insulin is not able to properly regulate blood glucose which normally is seen in adults and has direct relationship with obesity and lack of exercise (Foley *et al.*, 1998). About 90% of all diabetics have type 2 diabetes generally treated by the use of medication and in some cases, insulin.

2.2 HYPERTENSIVE AND DIABETIC NEPHROPATHY

Diabetic nephropathy (DN) is usually defined as a high concentration of excreted protein in urine. During the initial stage there is not a significant increase in excretion of urine albumin. This stage is called the incipient DN. (Valmadrid *et al.*, 2000). However, in more advanced states, the condition is spelt out by the presence of albumin or protein in urine. According to Parving *et al.* (1982), diabetic nephropathy also called Kimmelstiel-Wilson syndrome or interpapillary glomerulonephritis, is a clinical syndrome presenting with albumin in urine (>300 mg/day or >200 mcg/min), constant and invariable reduced glomerular filtration rate and arterial high blood pressure. Albuminuria should be established twice with 3 months intervals. Diabetic

nephropathy (DN) is the cause of chronic renal disease in patients initiating renal replacement therapy in the United States (USRD, 2003). The disease is associated with increased hypertension and cardiovascular mortality (Bruno and Gross, 2000).

In advanced and non-advanced countries Diabetes mellitus accounts for most of the cases resulting in chronic renal failure. (Mogensen and Christensen, 1984). Complications that arise as a result of having type 1 and 2 diabetes lead to Diabetic nephropathy. (Viberti *et al.*, 1982). According to Levey *et al.* (2000), there are 5 stratifications in developing diabetic nephropathy.

About one out of three Americans are hypertensive. This comes with its associated risk of heart related diseases. (Fields *et al.*, 2004). The Prevalence of hypertension has been reported to range from 19.3% to 54% in Ghana (Commodore-Mensah *et al*, 2014). Patients with hypertension are highly susceptible to co-exist with diabetes these people usually develop CKD. Much attention has been shifted on ways to bring down the development of diabetes among people with hypertension (Chobanian *et al.*, 2003).

2.2.1 Mechanisms by which diabetes causes Nephropathy

As a result of vascular defects that are associated with diabetes, one major complication that sets in is Diabetic nephropathy. (Haenggi *et al.*, 1999). Schlipak (2010) stated that progression to ESRD which is the terminal stage of chronic renal disease is mainly caused by diabetes mellitus. Chronic renal diseases which occur as a result of diabetes pass through progressive stages

The mechanism by which diabetes causes chronic renal disease is very complex.

During the initial stage, there is a high concentration of blood glucose in diabetics. This raises high inflammatory mediators within the kidney (Bauer et al., 2008). These

mediators cause the glomerulus to filter albumin and these get excreted in urine. High blood glucose concentration causes some proteins to bind to the glomerulus which leads to scarring of the glomerulus membrane. This condition develops over a period of years. (Davey, 2006).

As the localized scarring process worsens, the kidneys after a period of time lose their ability to filter blood. This results from the replacement of healthy tissues with scarred tissues. (Eckardt *et al.*, 2009). This gradual 'failing' of the kidneys progressively results in end-stage kidney failure. According to Brion et al. (1986), increased excretion of albumin in urine (where the excreted levels of albumin into the urine is between 30-300 mg/day) is the initial sign noticed in diabetics with renal conditions. This initial sign when not managed well over a period of time gives rise to excretion of protein in urine. (Gault *et al.*, 1992). Once proteinuria (where the amount of albumin that leaks into the urine is more than 300 mg per day) occurs, it is not reversible and, therefore, initiates the start of an insidious functional decline in the kidneys leading to terminal renal failure. Diabetic nephropathy leads to the glomerulus being damaged. This allows more proteins to be excreted in urine. The gradual damage of the kidneys over a period of time leads to the inability of the kidneys to function well and renal failure sets in. (Levey *et al.*, 2006) as depicted in
Fig. 2.1 below.

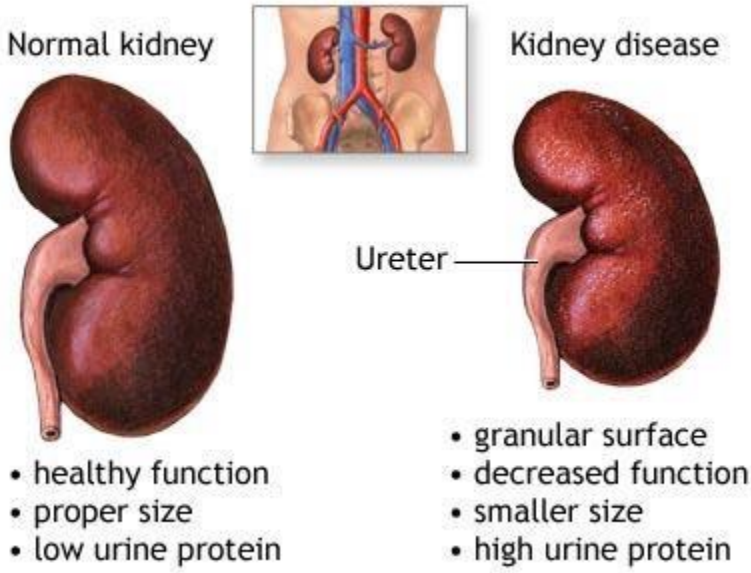

Figure 2.1: Healthy (left) and diseased (right) kidneys (Levey et al., 2006)

2.2.2 Mechanism by which hypertension causes nephropathy

Hypertension is defined as blood pressure that is too high. Hypertension ranks second to diabetes as a contributory factor to ESRD. Most people with chronic renal diseases regardless of the precipitating factors tend to develop hypertension (About 80% of people with chronic kidney conditions have hypertension setting in) (Toto, 2005). This is caused by two main things. When the kidneys are damaged and thus result in malfunctioning of the kidneys, regulation of fluids and electrolytes become poor leading to retention of fluid in the body. The second results from a series of processes called the Renin angiotensin system (RAS). Renin is involved in the control and regulation of blood pressure. When renin is released it activates RAS which leads to the blood vessels becoming occluded. When this happens there is a reduced flow of blood to the glomerulus and this stimulates the release of more renin leading to an elevated blood pressure in the body. In a person with CKD there is a reduced flow of blood to the glomerulus. This is the reason why people with CKD often develop hypertension even in the absence fluid retention. (Mathew *et al.,* 2007). However, the mechanism of

action of some drugs for hypertension is to block the Renin angiotensin system and this is more potent with regards to CKD management. (Levey *et al.,* 2009).

2.3 CHRONIC KIDNEY DISEASE

Chronic renal disease is made up of conditions that have an impact on the kidneys. These have the ability to slow down the functions of the kidneys gradually and also allow complications to set in as a result of impaired functions of the kidneys (Levey *et al.*, 1999). Harris *et al.* (1998) also defined CKD as an occurrence where the kidneys are damaged and its functions decreased for a period of 3 months or greater. A thematic flowchart of CKD is shown in Figure 2.2 as proposed by Thomas *et al.* (2008).

The decline in kidney function results in a gradual decrease in mineral homeostasis, with an accompanying changes in levels of calcium and phosphorous in tissues and serum. There is also a change in the concentrations of hormones. (Harmoinen *et al.*, 2003). Such hormones are inclusive of parathyroid hormone (PTH), 25hydroxyvitamin D (25(OH) D), 1, 25-dihydroxyvitamin D (1, 25(OH) 2D), and other vitamin D metabolites, fibroblast growth factor-23 (FGF-23), and growth hormone. At CKD stage 3, there is a decrease capacity of the kidneys to effectively cause phosphate to be excreted, resulting in high phosphate levels, high PTH levels, and low 1,25(OH)2D with corresponding increase in the concentrations of fibroblast growth factor-23 (Hojs *et al.*, 2004).

The disease develops over a long period as a result of damages to the kidneys. According to Mussap et al. (2002), CKD has multiple causes and the loss of function of the kidneys is mostly not reversible and can lead to morbidity. In certain situations, dialysis or kidney organ exchange are needed. In recent times more knowledge has been acquired concerning the dynamics of CKD and this has shown that the condition is

prevalent than previously known. (Chantrel *et al.*, 2000; Perlemoine *et al.*, 2003). Chronic renal conditions are more common in people of advanced age. This has the potential of increasing in a population (Burkhardt *et al.*, 2004).

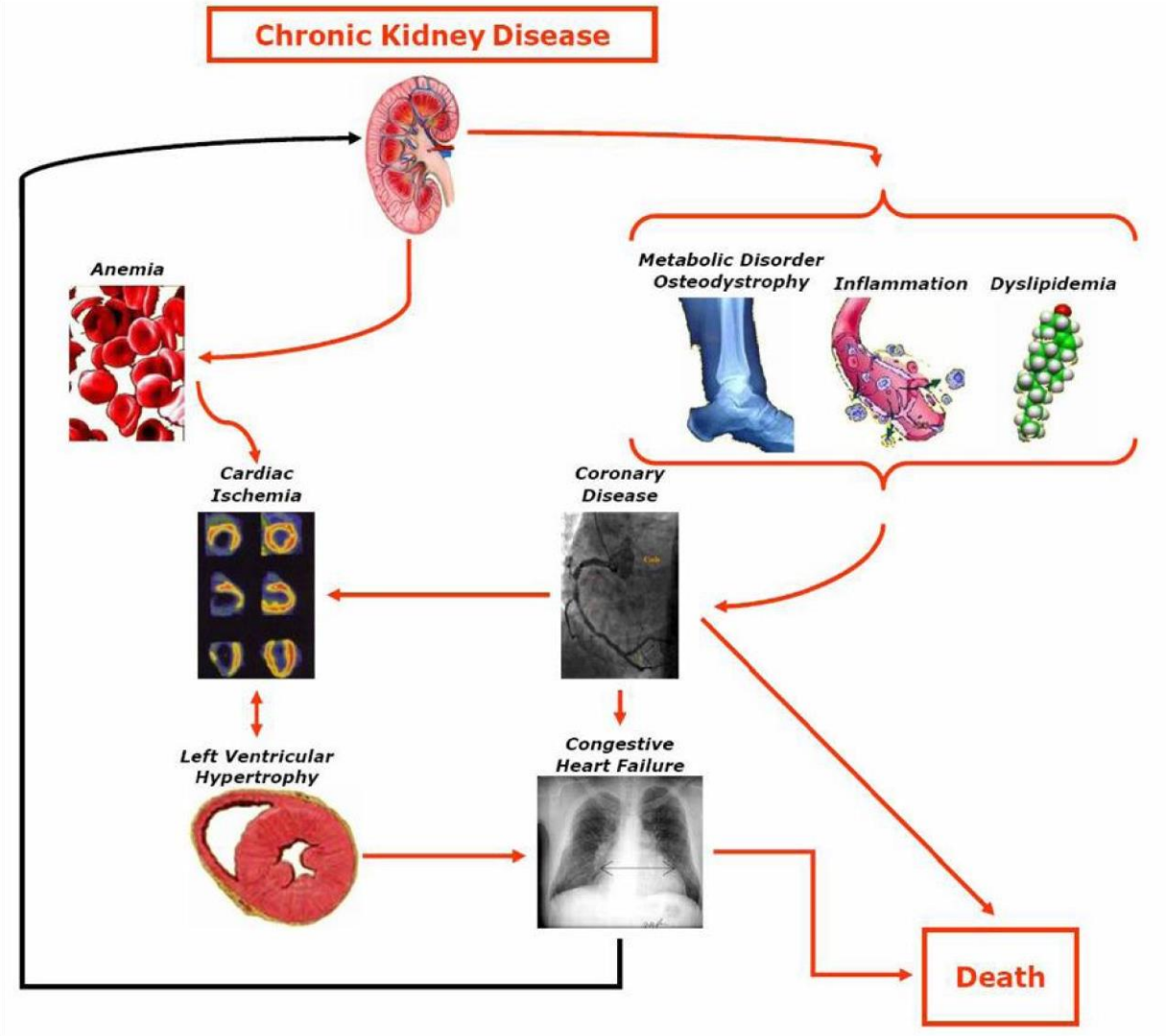

Figure 2.2: Interplay of processes secondary to chronic kidney disease leading to cardiovascular disease and death. Red arrows: Pathogenetic pathways; black arrow: Feedback loop; kidney disease worsened by heart failure (Source: Thomas et al., 2008).

2.3.1 Risk Determinants to Chronic Kidney Diseases

There are some determinants when present in a person will precipitate the development of a disease condition later in life. Some of these determinants are history of families and age and these cannot be controlled, regressed or prevented (Keith *et al.*, 2004). Instances where blood glucose concentrations and blood pressure are managed to their minimum levels, kidney function is aided. Some of the risk factors are as follow:

2.3.2 High Blood Pressure (Hypertension)

High blood pressure exerts more force on the walls of the blood vessels with the kidneys inclusive and thus, regarded as the number two cause of kidney failure (National Collaborating Centre for Chronic Conditions, 2008). Weight control, exercise, and medications which control blood pressure have the high chance of preventing or reducing the progression from kidney disease to kidney failure (Eknoyan, 2007).

2.3.3 Diabetes

According to Crowe et al. (2008), diabetes is the number one risk factor for the development of renal disease with a representation of about 40%. However, the primary risk factor for renal failure is type 2 diabetes compared type 1 diabetes.

2.3.4 Blockages

When the lower urinary tract system is not formed well, and also when scars develop as a result of infections, there is a backflow of urine to the kidneys. This situation causes the kidneys to malfunction. Thrombus formation and cholesterol plaques cause a narrowing of the blood vessels in the kidneys. This leads to a reduced perfusion of blood to the kidneys thus causing destruction to the kidneys. (Taylor *et al.*, 2005) (Weiner *et al.*, 2004).

2.3.5 Overuse of painkillers and allergic reactions to antibiotics

One of the causes of kidney failure is the inflammation of the kidneys known as interstitial nephritis. This results from the abuse of painkillers, side effects of antibiotics such as erythromycin and anaphylactic reactions due to antibiotic intake.
(Eknoyan, 2007) (National Kidney Foundation, 2002).

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2.3.6 Drug Abuse

Nephrotoxic drugs cause damage to the kidneys. Substances such as cocaine and heroin have negative impact on the kidneys which can cause the kidneys to fail.
(Burkhardt *et al.*, 2004).

2.3.7 Family History of Kidney Disease

People belonging to a family with more of its members suffering from CKD that requires hemodialysis are at a high risk of developing CKD (National Kidney Foundation, 2002). Inherited disease such as the formation of many cysts in the kidneys result in tissues of the kidneys being replaced by these cysts and subsequently results in CKD and kidney failure. There is therefore the need for periodic medical screening of members from such families.

2.3.8 Age

The ability of the kidney to work dwindles in people of advanced ages therefore is a major risk determinant for CKD. When GFR decreases, it increases the risk of mortality with progression of age. However, evidence in studies with larger populations show otherwise. (Foley *et al.*, 1998). A fall in GFR is the number one cause of mortality in kidney conditions, heart related conditions and the aged. (Keith *et al.*, 2004). A research study carried out in the year 2001 by the Department of Veterans Affairs with a

onetime assay for creatinine showed that about 50% of the patients with advanced ages recorded eGFR less than 60 ml/min per 1.73 m^2. The percentage was lower in young age groups (Eknoyan *et al.*, 2007). However, when compared, deaths due to a decrease in GFR was lower in the patients with advanced age groups.

2.3.9 Symptoms of Chronic Kidney Diseases

There are several symptoms of CKD exhibited by infected patients of which the most prominent ones are as follow:

1. Changes in urination

Patients suffering from CKD generally experience change in urination (Nilsson *et al.*, 1994). Thus, there is polyuria, passing out of foamy urine, greater urine volume than usual, passage of dark colored urine and feeling pressured or difficulties in the passage of urine.

2. Swelling in the legs, ankles, feet, face, and/or hands

A reduced kidney function causes accumulation of fluid in the body tissues due to the failure of the kidneys to excrete extra fluids.

3. Fatigue

Normal kidneys produce erythropoietin a hormone that regulates erythropoietic activities leading to increased production of red cells. A decreased kidney function reduces the production of the hormone which ultimately leads to reduced red cells and oxygen produced. (Le Bricon *et al.*, 1999).

4. Metallic taste in mouth/ammonia breath

When blood accumulate wastes it comes with a change in how food tastes and also odour in the mouth. There is also an incidence of loss of appetite for meat and lose of body weight.

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2.3.10 Complications of Chronic Kidney Disease

2.3.10.1 Neuropathy

Kidney pathology aggravation is often seen in patients whose kidneys have failed. This is associated with nervous system dysfunction, singular or multiple damages to the brain and spinal cord nerves, sleep disorders and abnormal brain function. (Warram *et al.,* 1996). Regardless of the type of kidney disease, a failure of the kidneys is manifested by brain neuropathy. However certain diseases which cause kidney pathology also independently cause neuropathy. Some of these are the failure of the kidneys to form well, diabetes, buildup of amyloids in the body and an autoimmune disease that attack the organs and tissues. (Barratt *et al.,* 1970). The mechanism of how accumulation of wastes in the blood causes a decrease of nerve function is not well fashioned out. The inability of electrical impulses to move through the nerves has been correlated to levels of certain biomarkers like creatinine and urea in the blood

2.3.10.2 Cardiovascular risk

It has been established that ESRD poses a great cardiovascular risk. The mortality rates as a result of the cardiovascular consequences is 10-100x more in people on dialysis than individuals matched for sex and age in a generalized population (Foley *et al.,* 1998). The risk of cardiovascular disease as a result of kidney damage rises early as kidney disease progresses than was initially imagined. It is well documented that

cardiovascular risk is increased by even mild to moderate degrees of kidney impairment. Several of the established risk determinants recorded in the general population increase the risk of cardiovascular disease in persons with chronic renal conditions. In reality, numerous heart related risk determinants are more evident in CKD patients as compared to persons with functional kidneys. Additionally, non-established risk determinants which are peculiar to people with CKD also add up to the cardiovascular disease load. Hypertension, a traditional cardiovascular risk determinant, adds to the heart related risk connected to CKD. Investigations have shown that patients with hypertension are more susceptible to chronic heart related events especially in people with CKD staging of 2 and 3 (Muntner *et al.,* 2002). Cardiovascular fatalities in patients on dialysis are most often related to systolic pressure than either pulse or diastolic pressure (Port *et al.,* 1999). Nonetheless, there is an association that exist. Fluctuating blood pressure are apparently associated with increased rate of mortality among stage 5 CKD patients.

2.3.10.3 Dyslipidemia

It is well known that patients with impaired renal function exhibit significant changes in lipoprotein metabolism which in their most severe forms may result in severe dyslipidemia. Dyslipidemia in patients with CKD is commonly characterized by high triglyceride levels with low or normal levels of LDL and total cholesterol (Tsimihodimos *et al.,* 2011). Excessive production of triglyceride by the hepatocytes as a result of increase influx of free fatty acids in the liver results in hypertriglyceridemia (Sowers, 2007). Many studies have shown in patients with CKD an inverse relation between creatinine and triglyceride.

2.3.11 Chronic Kidney Disease Staging

Stage I: Hypertrophic hyper filtration

This stage is characterized by normal functioning of the kidneys. The anatomy of the kidneys is intact. It spans from the beginning of the condition to about 5 years of the disease. The blood pressure and GFR are all within normal range.

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Stage II: The dormant stage

It is characterised by anatomical changes in the kidneys. This is noticed three years after the beginning of the condition. At this stage, the patient is asymptomatic. GFR is mostly within reference values and many people do not progress to other stages from here.

Stage III: The micro albuminuria stage

In this stage patients become symptomatic with the presence of small amount of albumin in urine. There is an anatomical change in the kidneys. It starts
6 years after the onset of the disease. There is often an elevated blood pressure. (Perkins and Krolewski, 2009).

Stage IV: Chronic renal failure

At this stage of the disease, there is the development of proteinuria (albumin > 350 mg/dU), decline in GFR less than 60 ml/min/1.73 m^2 and high pressure levels in the blood. (Young *et al.*, 2003).

Stage V: End Stage Renal Disease (GFR < 15 ml/min/1.73 m^2)

This is the last stage in the stratification of CKD where there is a highly reduced GFR, abnormally high blood pressure, extensive kidney damages and proteinuria. The end

result is death if patients do not undergo an organ transplant or put on dialysis. Only few patients who develop chronic kidney disease progress to this stage. It is the most fatal. (Bruno and Gross, 2000) (Valmadrid *et al.*, 2000). The stages are diagrammatically shown in Table 2.1.

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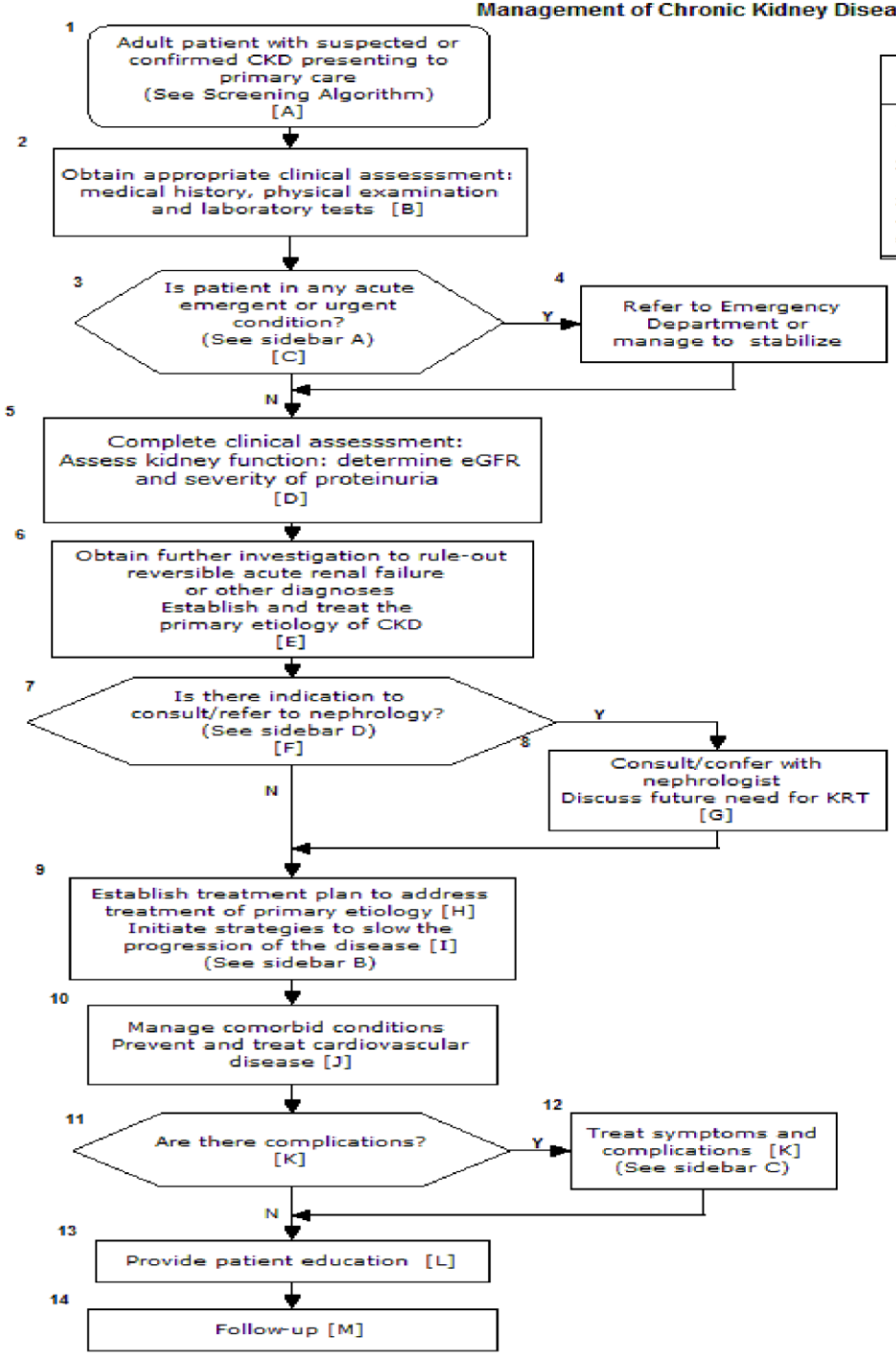
Stages of Chronic Kidney Disease		
Stage	**Description**	**GFR ($mL/min/1.73\ m^2$)**
1	Kidney Damage with Normal or ↑ GFR	≥ 90
2	Kidney Damage with Mild ↓ GFR	60-89
3	Moderate ↓ GFR	30-59
4	Severe ↓ GFR	15-29
5	Kidney Failure	<15 or Dialysis

Table 2.1: Stages of chronic kidney disease development (Source: Young *et al.*, 2003).

2.3.12 Control of Chronic Kidney Disease

Chronic kidney disease is disease which develops in many people worldwide. Although, CKD is more prevalent, it could be controlled or prevented by quitting smoking, avoiding certain pain relief medications, doing regular exercise, consuming food rich in plant protein and controlling blood sugar level (Fig. 2.3) (Stevens and Levey, 2005).

VA/DoD Clinical Practice Guideline
Management of Chronic Kidney Disease



Stage	eGFR (ml/min/1.73m2)	Description
1	> 90	Kidney damage with normal or increased GFR
2	60-89	Kidney damage with mildly decreased GFR
3	30-59	Moderately decreased GFR
4	15-29	Severely decreased GFR
5	< 15 or dialysis	Kidney failure

Sidebar A: Urgent/Emergent Conditions

- Acute unexplained decline in kidney function
- Heart failure/volume overload
- Hyperkalemia (potassium ≥6 mEq/L)
- Signs or symptoms of uremia

Sidebar B: Strategies to Slow Progression

1. Control of hypertension
2. Use of ACEI/ARB
3. Control of hyperglycemia
5. Avoid toxic drugs
6. Smoking cessation
7. Control of dyslipidemia

Sidebar C: Prevention and Treatment of Complications

- Metabolic disorders:
  - -potassium balance
  - -calcium, phosphate balance
  - -acidosis
- Anemia
- Volume overload
- Overuse of renally excreted drugs
- Nutrition

Sidebar D: Indications for Nephrology Consultation

1. eGFR <30 ml/min/1.73m^2
2. Rapid decline of GFR
3. Severe complications of CKD (e.g., recalcitrant anemia, calcium or phosphorus abnormalities)
4. Nephrotic range proteinuria (>3.5 grams/24 hours)
5. Underlying cause of CKD is unclear after basic work-up
6. Kidney biopsy is indicated
7. Patient's level of disease exceeds the level of comfort of the primary care provider

2.4 GLOMERULAR FILTERATION RATE

Glomerular filtration rate (GFR) is the volume of fluid filtered from the renal (kidney) glomerular capillaries into the Bowman's capsule per unit time. Central to the physiologic maintenance of GFR is the differential basal tone of the afferent and efferent arterioles (Mathew *et al.,* 2007). Glomerular filtration rate (GFR) is equal to the Clearance Rate when any solute is freely filtered and is neither reabsorbed nor secreted by the kidneys. The rate therefore measured is the quantity of the substance in the urine that originated from a calculable volume of blood. Relating this principle to the below equation - for the substance used, the product of urine concentration and urine flow equals the mass of substance excreted during the time that urine has been collected. This mass equals the mass filtered at the glomerulus as nothing is added or removed in the nephron. Dividing this mass by the plasma concentration gives the volume of plasma which the mass must have originally come from, and thus the volume of plasma fluid that has entered Bowman's capsule within the aforementioned period of time. The GFR is typically recorded in units of *volume per time*, for instance, milliliters per minute mL/min. Compare to filtration fraction.

$$GFR = \frac{\text{Urine Concentration} \times \text{Urine Flow}}{\text{Plasma Concentration}}$$

There are several different techniques used to calculate or estimate the glomerular filtration rate (GFR or eGFR). The GFR equation in page 26 only applies for GFR calculation when it is equal to the Clearance Rate.

2.4.1 Overview of markers for GFR

Glomerular filtration rate (GFR) is difficult to measure in clinical practice (Perrone *et al.,* 1990). Laboratory markers for the estimation of GFR which are synthesized endogenously,

have regular production rate, are eliminated freely by the glomerulus, and not reabsorbed stand a high chance of classifying CKD appropriately (Burkhardt *et al.,* 2002). Creatinine clearance (CrC) using a 24-hour urine sample and serum creatinine (Cr) are the most commonly used parameters to estimate GFR in clinical practice although not the most accurate. However, there are limitations to their use. Creatinine could be affected by non-renal factors like muscle mass, protein intake, inflammatory illness, or hepatic disease (Oddoze *et al.*, 2001). Moreover, Cr is partially secreted by renal tubules and frequently overestimates GFR (O'Riordan *et al.*, 2003). On the other hand, CrC requires urine collection over a 24-hour period with a steady-state situation.

Cystatin C has become an established marker for Glomerular Filtration Rate (GFR), offering fast and early detection of reduced renal function and providing essential information for patient management. Accurate and precise estimation of GFR is highly beneficial both in terms of assessing kidney function, monitoring patients with renal diseases, and to find appropriate treatment (Perrone *et al.,* 1990). The extensive use of cystatin C as a clinical marker for estimation of GFR has increased the demand for standardized protocols.

Accurate assessment of GFR is needful for interpreting symptoms and signs, for drug dosing; for detecting and managing chronic kidney disease and assessing prognostic outcomes. A reduction in GFR to less than 60 ml per minute per 1.73 m^2 for 3 months or longer is a diagnostic criterion for chronic kidney disease and is associated with an increased risk of adverse outcomes, including death (Grubb *et al.*, 2005). A study conducted by Mahajan *et al*. (2005) using the combined creatinine–cystatin C equation and the average of the new cystatin C equation and the creatinine equation was similar to that with the individual creatinine and cystatin C equations, but they had greater precision and accuracy and resulted in more accurate classification of measured GFR.

However, since cystatin C is less affected by muscle mass and diet than creatinine, it has been widely anticipated that cystatin C could provide a more accurate estimate of GFR than creatinine (O'Riordan *et al.,* 2003).

Nevertheless, GFR estimates based on equations that use cystatin C as the sole filtration marker are not more accurate than creatinine based estimates, suggesting that unmeasured and largely unknown non-GFR determinants of cystatin C are similar in magnitude to those of creatinine (Levey *et al.,* 2007). The advantage of the cystatin C–based equation over the creatinine–based equation is that it is less subjected to the effects of age, sex, and race. Given the difficulties in assigning race and the lack of information about race in laboratory and administrative databases, a GFR estimating equation that does not require race may be more generalizable across populations and could greatly facilitate the use of estimated GFR in clinical practice, research, and public health programs (Stevens *et al.,* 2008a). In addition, the equation that combines creatinine and cystatin C provides the most precise and accurate estimate of GFR across the range of GFRs and in subgroups based on demographic and clinical characteristics.

According to Efron and Tibshirani (1993), the gold standard methods of estimating GFR require measurement of an ideal filtration marker. These markers should be freely filtered by the glomerulus, should not be bound to plasma proteins, must be excreted unchanged and not be subject to either tubular secretion or absorption. Commonly used markers include inulin, 51Cr-EDTA, 125I-iothalamate and iohexol. Albuminuria remains the only biomarker acceptable for diagnostic purposes, although some growth factors are expected to replace albuminuria in future. It is known that values of TGF beta, vascular endothelial growth factor (VEGF), and CTGF are increased in the plasma and urine of patients with diabetic nephropathy (Grubb *et al.,*

2005).

2.4.2 GFR measurement Using Inulin

The GFR can be determined by injecting inulin or the inulin-analog sinistrin into the plasma. Since both inulin and sinsitrin, are neither reabsorbed nor secreted by the kidney after glomerular filtration, their rate of excretion is directly proportional to the rate of filtration of water and solutes across the glomerular filter. Compared to the MDRD formula, the inulin clearance slightly overestimates the glomerular function. In early stage renal disease, the inulin clearance may remain normal due to hyperfiltration in the remaining nephrons. Incomplete urine collection is an important source of error in inulin clearance measurement (Mathew *et al.,* 2007).

2.4.3 Creatinine-based approximations of GFR

In clinical practice, however, creatinine clearance or estimates of creatinine clearance based on the serum creatinine level are used to measure GFR. Creatinine is produced naturally by the body (creatinine is a breakdown product of creatine phosphate, which is found in muscle). It is freely filtered by the glomerulus, but also actively secreted by the peritubular capillaries in very small amounts such that creatinine clearance overestimates actual GFR by 10-20% (National Kidney Foundation, 2002). This margin of error is acceptable, considering the ease with which creatinine clearance is measured. Unlike precise GFR measurements involving constant infusions of inulin, creatinine is already at a steady-state concentration in the blood, and so measuring creatinine clearance is much less cumbersome. However, creatinine estimates of GFR has its limitations. All of the estimating equations depend on a prediction of the 24hour

creatinine excretion rate, which is a function of muscle mass. Some of the equations, the Cockcroft and Gault do not correct for race and, therefore, predicts that black men and women have higher amount of muscle mass than Caucasian; hence, they will have higher serum creatinine levels at any level of clearance.

A common mistake made when just looking at serum creatinine is the failure to account for muscle mass. Hence, an older woman with a serum creatinine of 1.4mg/dl may actually have a moderately severe degree of renal insufficiency, whereas a young muscular male can have a normal level of renal function at this serum creatinine level (Mathew *et al.,* 2007). Creatinine-based equations should be used with caution in cachectic patients and patients with cirrhosis. They often have very low muscles mass and a much lower creatinine excretion rate than predicted by the equations in page 32, such that a cirrhotic patient with a serum creatinine of 0.9 mg/dl may have a moderately severe degree of rental insufficiency (Garasto *et al.,* 2014).

2.4.4 Creatinine Clearance- C_{Cr}

One method of determining GFR from creatinine is to collect urine (usually for 24 hours) to determine the amount of creatinine that was removed from the blood over a given time interval (National Institute of Diabetes and Digestive and Kidney Diseases, 2012). If one removes, 1440 mg in 24 hours, this is equivalent to removing 1 mg/min. If the blood concentration is 0.01 mg/mL (1 mg/dl), then one can say that 100 mL/min of blood is being "cleared" of creatinine, since, to get 1 mg of creatinine, 100 mL of blood containing 0.01 mg/mL would need to have been cleared.

Creatinine clearance (C_{Cr}) is calculated from the creatinine concentration in the collected urine sample (U_{Cr}), urine flow rate (v*dt*), and the plasma concentration (P_{Cr}).

Since the product of urine concentration and urine flow rate yields creatinine excretion rate, which is the rate of removal from the blood, creatinine clearance is calculated as removal rate per min ($U_{Cr} \times V dt$) divided by the plasma creatinine concentration (Matsushita et al., 2010). This is commonly represented mathematically as

$$C_{Cr} = \frac{U_{Cr} \times \dot{V}}{P_{Cr}}$$

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Example: A person has a plasma creatinine concentration of 0.01 mg/ml and in 1 hour produces 60ml of urine with a creatinine concentration of 1.25 mg/mL.

$$C_{Cr} = \frac{1.25mg/mL \times \frac{60mL}{60min}}{0.01mg/mL} = \frac{1.25mg/mL \times 1mL/min}{0.01mg/mL} = \frac{1.25mg/min}{0.01mg/mL} = 125mL/min$$

The common procedure involves undertaking a 24-hour urine collection. The bladder is emptied and every urine voided after that is collected over 24 hours. A blood sample is analysed for comparison. The urinary flow rate is still calculated per minute, hence:

$$C_{Cr} = \frac{U_{Cr} \times \text{24-hour volume}}{P_{Cr} \times 24 \times 60mins}$$

To allow comparison of results between people of different sizes, the C_{Cr} is often corrected for the body surface area (BSA) and expressed compared to the average sized man as mL/min/1.73 m^2. While most adults have a BSA that approaches 1.7 (1.6-1.9), extremely obese or slim patients should have their C_{Cr} corrected for their *actual* BSA.

$$C_{Cr-corrected} = \frac{C_{Cr} \times 1.73}{BSA}$$

BSA can be calculated on the basis of weight and height.

Twenty-four-hour urine collection to assess creatinine clearance is no longer widely performed, due to difficulty in assuring complete specimen collection. To assess the adequacy of a complete collection, one always calculates the amount of creatinine excreted over a 24-hour period. This amount varies with muscle mass, and is higher in

young people vs. old, in blacks vs. whites, and in men vs. women. An unexpectedly low or high 24-hour creatinine excretion rate voids the test. Nevertheless, in cases where estimates of creatinine clearance from serum creatinine are unreliable, creatinine clearance remains a useful test. These cases include "estimation of GFR in individuals with variation in dietary intake (vegetarian diet, creatine supplements) or muscle mass (amputation, malnutrition, muscle wasting), since these factors are not specifically taken into account in prediction equations."

2.4.5 Estimated creatinine clearance rate (eC_{Cr}) using Cockcroft-Gault formula

A number of formulae have been devised to estimate GFR or C_{cr} values on the basis of serum creatinine levels (Stevens *et al.*, 2008a). A commonly used surrogate marker for estimate of creatinine clearance is the Cockcroft-Gault (CG) formula, which in turn estimates GFR in ml/min. It is named after the scientists who first published the formula, and it employs serum creatinine measurements and a patient's weight to predict the creatinine clearance (Cockcroft and Gault, 1976). The formula, as originally published, is:

$$eC_{Cr} = \frac{(140 - \text{Age}) \times \text{Mass (in kilograms)} \times [0.85 \; if \; Female]}{72 \times \text{Serum Creatinine (in mg/dL)}}$$

This formula expects weight to be measured in kilograms and creatinine to be measured in mg/dl, as is standard in the USA. The resulting value is multiplied by a constant of 0.85 if the patient is female. This formula is useful because the calculations are simple and can often be performed without the aid of a calculator.

When serum creatinine is measured in µmol/L:

$$eC_{Cr} = \frac{(140 - \text{Age}) \times \text{Mass (in kilograms)} \times Constant}{\text{Serum Creatinine (in } \mu\text{mol/L)}}$$

Where *Constant* is *1.23* for men and *1.04* for women.

One interesting feature of the Cockcroft and Gault equation is that it shows how dependent the estimation of CCr is based on age. The age term is (140 - age). This means that a 20-year-old person (140-20 = 120) will have twice the creatinine clearance as an 80-year-old (140-80 = 60) for the same level of serum creatinine (120 is twice as great as 60). The C-G equation assumes that a woman will have a 15% lower creatinine clearance than a man at the same level of serum creatinine.

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2.4.6 Estimated GFR (eGFR) using Modification of Diet in Renal Disease (MDRD) formula

The most recently advocated formula for calculating the GFR is the one that was developed by the *Modification of Diet in Renal Disease Study Group* (Rule et al., 2004). Most laboratories in Australia and The United Kingdom now calculate and report the MDRD estimated GFR along with creatinine measurements and this forms the basis of chronic kidney disease. The adoption of the automatic reporting of MDRD-eGFR has been widely criticized (Fatma *et al.*, 2013).

The most commonly used formula is the "4-variable MDRD," which estimates GFR using four variables: serum creatinine, age, ethnicity, and gender. The original MDRD used six variables with the additional variables being the blood urea nitrogen and albumin levels. The equations have been validated in patients with chronic kidney disease; however, both versions underestimate the GFR in healthy patients with GFRs over 60 mL/min. The equations have not been validated in acute renal failure.

For creatinine in µmol/L:

$$\text{eGFR} = 32788 \times \text{Serum Creatinine}^{-1.154} \times \text{Age}^{-0.203} \times [1.210\ if\ Black] \times [0.742\ if\ Female]$$

For creatinine in mg/dl:

$$\text{eGFR} = 186 \times \text{Serum Creatinine}^{-1.154} \times \text{Age}^{-0.203} \times [1.210\ if\ Black] \times [0.742\ if\ Female]$$

Creatinine levels in µmol/L can be converted to mg/dl by dividing them by 88.4. The 32788 number above is equal to $186 \times 88.4^{1.154}$.

A more elaborate version of the MDRD equation also includes serum albumin and blood urea nitrogen (BUN) levels:

$$\text{eGFR} = 170 \times \text{Serum Creatinine}^{-0.999} \times \text{Age}^{-0.176} \times [0.762\ if\ Female] \times [1.180\ if\ Black] \times \text{BUN}^{-0.170} \times \text{Albumin}^{+0.318}$$

Where, the creatinine and blood urea nitrogen concentrations are both in mg/dl. The albumin concentration is in g/dl.

These MDRD equations are only used when the serum creatinine measurements have not been calibrated to isotope dilution mass spectrometry (IDMS) (Twomey and Reynolds 2006). When IDMS-calibrated serum creatinine is used (which is about 6% lower), the above equations should be multiplied by 175/186 or by 0.94086. Since these formulae do not adjust for body mass, they (relative to the Cockcroft-Gault formula) underestimate eGFR for heavy people and overestimate it for underweight people.

2.4.7 Estimated GFR (eGFR) using the CKD-EPI formula

The CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) formula was published in May 2009. It was developed in an effort to create a formula more accurate than the MDRD formula, especially when actual GFR is greater than 60 mL/min per 1.73 m^2.

Researchers pooled data from multiple studies to develop and validate this new equation (Schwartz *et al.*, 2009). They used 10 studies that included 8254 participants, randomly

using 2/3 of the data sets for development and the other 1/3 for internal validation. Sixteen additional studies, which included 3896 participants, were used for external validation.

The CKD-EPI equation performed better than the MDRD (Modification of Diet in Renal Disease Study) equation, especially at higher GFR, with less bias and greater accuracy. When looking at NHANES (National Health and Nutrition Examination Survey) data, the median estimated GFR was 94.5 mL/min per 1.73 m2 vs. 85.0 mL/min per 1.73 m2, and the prevalence of chronic kidney disease was 11.5% versus 13.1%.

The CKD-EPI equation, expressed as a single equation, is:

$$\mathrm{eGFR} = 141 \times \min(\mathrm{SCr}/k,1)^{a} \times \max(\mathrm{SCr}/k,1)^{-1.209} \times 0.993^{Age} \times [1.018\ if\ Female] \times [1.159\ if\ Black]$$

Where, SCr is serum creatinine (mg/dl), k is 0.7 for females and 0.9 for males, a is 0.329 for females and -0.411 for males, min indicates the minimum of SCr/k or 1, and max indicates the maximum of SCr/k or 1.

A clearer version may be as follows: For creatinine (IDMS calibrated) in mg/dl:

Black Female

If serum creatinine (Scr) <= 0.7

$$\mathrm{eGFR} = 166 \times (\mathrm{SCr}/0.7)^{-0.329} \times 0.993^{Age}$$

If serum creatinine (Scr) > 0.7

$$\mathrm{eGFR} = 166 \times (\mathrm{SCr}/0.7)^{-1.209} \times 0.993^{Age}$$

Black male

If serum creatinine (Scr) <= 0.9

$$\mathrm{eGFR} = 163 \times (\mathrm{SCr}/0.9)^{-0.411} \times 0.993^{Age}$$

If serum creatinine (Scr) > 0.9

$$\mathrm{eGFR} = 163 \times (\mathrm{SCr}/0.9)^{-1.209} \times 0.993^{Age}$$

White or other race female

If serum creatinine (Scr) <= 0.7

$$\mathrm{eGFR} = 144 \times (\mathrm{SCr}/0.7)^{-0.329} \times 0.993^{Age}$$

If serum creatinine (Scr) > 0.7

$$\mathrm{eGFR} = 144 \times (\mathrm{SCr}/0.7)^{-1.209} \times 0.993^{Age}$$

White or other race male

If serum creatinine (Scr) <= 0.9

$$\mathrm{eGFR} = 141 \times (\mathrm{SCr}/0.9)^{-0.411} \times 0.993^{Age}$$

If serum creatinine (Scr) > 0.9

$$\mathrm{eGFR} = 141 \times (\mathrm{SCr}/0.9)^{-1.209} \times 0.993^{Age}$$

$$\mathrm{eGFR} = \exp(1.911 + 5.249/Serum\ Creatinine - 2.114/Serum\ Creatinine^2 - 0.00686 \times \mathrm{Age} - [0.205\ if\ Female])$$

$$\mathrm{eGFR} = \frac{k \times Height}{Serum\ Creatinine}$$

Where *k* is a constant that depends on muscle mass, which itself varies with a child's age:

In first year of life, for pre-term babies K = 0.33 and for full-term infants K = 0.45 For infants and children of age 1 to 12 years, K = 0.55.

2.4.8 Serum Creatinine

Creatinine is a waste product that is made when the body breaks down protein and also, when muscles are injured (Lawrence *et al.*, 1998). A high serum (blood) creatinine level then implies kidney damage. However, creatinine levels vary somewhat, even when the kidneys work normally (Thomas *et al.*, 2008). Creatinine levels tend to be higher in

men and people with large muscles. Measuring creatinine is therefore, a step to finding the level of kidney function. Historically, measurement of creatinine or urea in serum or plasma has been used to assess kidney function. Both are convenient but insensitive (about 50% of the kidneys have to be destroyed before a significant rise in serum creatinine becomes apparent) (Poggio *et al.,* 2005). In addition, serum concentrations of creatinine are affected by various analytical interferences, and depend critically on muscle mass, for example, a serum creatinine concentration of 130 umol/l might be normal in one individual but require further investigation in another.

Other factors which affect creatinine concentrations include age, sex, ethnicity, body habitus and diet (Coresh et al., 2003). Diet may have a rapid and transient effect on creatinine concentration and there is evidence that consumption of cooked meat, in particular, may affect CKD categorization based on estimated glomerular filtration rate (eGFR).

2.4.9 Serum Cystatin C

Cystatin C is a nonglycosylated protein that belongs to cysteine protease inhibitors, the cystatin superfamily (Nordén *et al.,* 1987). These proteins play an important role in the regulation of proteolytic damage to the cysteine proteases. Cystatin C is produced at a constant rate by nucleated cells (Barrett *et al.,* 1986). It is found in relatively high concentrations in many body fluids, especially in the seminal fluid, cerebrospinal fluid and synovial fluid. Its low molecular weight (13.3 kDa) and positive charge at physiological pH levels facilitate its glomerular filtration. Subsequently, it is reabsorbed and almost completely catabolized in the proximal renal tubule (Keevil *et al.,* 2003). Therefore, because of its constant rate of production, its serum concentration is determined by glomerular filtration (Hoek *et al.,* 2003). Moreover, its concentration is not influenced by infections, liver diseases, or inflammatory diseases. The use of serum

cystatin C as a marker of GFR is well documented, and some authors have suggested that it may be more accurate than serum creatinine (Menon *et al.,* 2008).

Cystatin C is a 13 KD protein composed of 120 amino acid residues which produce in all nucleate cells of the body. Cystatin C is derived from a pro-protein containing cystatin C and a peptide signal, being this signal peptide is emphasis on its secretory action and also extracellular preventive operation of this protein (Barrett *et al.,* 1986). Cystatin C production rate is partly constant from two months old to seventy years old and proportional to the amount of Glomerular Filtration Rate (GFR). Unlike serum creatinine, rate of its production is not affected by muscular mass and sex. It is filtered easily from the glomerulus due to the small size and pure positive net charge, moreover, its concentration is equivalent to the plasma. This substance generally absorbed by proximal tubule and its appearance in the urine indicate a deficiency in proximal tubule. However, the potential limitations of cystatin C as a marker of GFR include lack of assay standardization, the requirement for a dedicated analytical system, and increased costs relative to serum creatinine (Mahajan *et al.,* 2005).

2.4.10 Serum Beta 2 Microglobulin

B2- Microglobulin (M.W 11815 dalton) is filtered at the glomerulus freely. Afterwards almost the entire substance is reabsorbed and broken down in the renal tubule. The plasma concentration in health is often low because it is filtered so freely (average 1.5 mg/L). The plasma concentration increases as the glomerular filtration rate declines reaching about 40 mg/l in terminal uremia. Plasma β- microglobulin concentration logarithm is linearly related to the logarithm of glomerular filtration rate through the whole range so that it serves as a good marker of renal dysfunction.

The plasma concentration of β-microglobulin is neither affected by muscle mass nor by the sex of an individual. Rise in plasma concentration could be due to increased production rather than reduced clearance in patients with some tumors and inflammatory diseases (Schardijn and Statius van Eps, 1987).

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2.5 THE ROLE OF LIFESTYLE AND KIDNEY DISEASE

Diet is a key to many of the 'factors' thought to promote and inhibit the formation of kidney diseases (Vimalachandra *et al.,* 2006). It has been reported that inappropriate dietary habits and lifestyle promote kidney stone formation (Salusky *et al.,* 1998). Ten to fifteen per cent of worldwide population therefore, experience kidney stone during their lifetime, many of whom have recurrence. Lifestyle therefore, play vital roles in the regulation of the occurrence of kidney diseases as explained below.

2.5.1 Increased fluid intake

An increase intake of fluids helps dilute urine which helps prevent the formation of kidney stones. (Atkins *et al.,* 2004). A fluid intake producing a consistent urine volume

of at least 2 litres per day really aid in the prevention of stone recurrence and studies by Sterrett *et al*. (2007) suggest a fluid intake of 2.5–3 litres per day.

2.5.2 Maintained adequate calcium intake

Studies of the effect of dietary calcium on stone recurrence rates have led to major changes in dietary advice. Epidemiological studies have shown an inverse relationship between dietary calcium and stone recurrence rates, possibly caused by calcium inhibiting the intestinal absorption of oxalate (Salusky *et al.,* 1998; Parfitt, 2003). This is supported by a recent study done by Fujii et al. (2007) which indicated that taking in a diet of high calcium and restricted oxalate, protein and salt had half as many stone recurrences as those on a low calcium diet.

2.5.3 Limited intake of animal protein

The effect of excess animal protein (purine) is most obvious for the uric acid-stone former (Freemont and Malluche, 2005b). Uric acid is a by-product of purine metabolism and excess animal protein creates urine with a high uric acid

concentration, which is a risk factor for uric acid stones. Animal protein also increases calcium in the urine and lowers citrate levels (an inhibitor of calcium stone formation) (Meschi *et al.,* 2006); thus there is a link between meat consumption and both uric acid and calcium stone formation. Giannini et al. (1999) suggested that protein from plant sources could therefore, be the best substitute without any negative consequence. A recent study highlighted the benefits of animal protein restriction on urinary markers of calcium stone formation (Taylor *et al.*, 2005; Curhan *et al.,* 2010). Restriction of animal protein intake to less than 80g per day therefore, has high prospects in reducing kidney diseases.

2.5.4 Maintained Phosphorus In-take

Maintaining normal serum phosphate levels is important for preventing renal bone disease and calcification of the soft tissue in people with CKD (Baggio *et al.,* 2000). Increase in blood phosphate is not usually seen until the later stages of CKD. Referral to a nephrologist is needed to optimize renal bone disease management through prescription of phosphorus binding medications and vitamin D derivatives (Taylor *et al.,* 2005).

2.5.5 Weight Management

The risk of serious health conditions such as kidney stones, diabetes, heart disease, arthritis, and stroke, as well as high blood pressure increases with body mass index (BMI) of 25 or higher (BMI = weight in kilograms divided by height in meters squared [kg/m2]) (Foley *et al.,* 1998). Overweight patients of BMI of 25 to 29.9 and obese patients of BMI of 30 or higher are usually prone to many diseases and thus, need to eat healthy diet and increase physical activity in order to lose weight and decrease the rate of diseases and infections.

2.5.6 Maintained Regular Exercise

Risk factors make it more likely that a disease would develop later. Some risk factors such as age or family history cannot be controlled (Keith *et al.,* 2004). However, other risk factors can be controlled to slow down or prevent diseases. Physical activities can help in controlling blood pressure and blood sugar levels.

CHAPTER 3

MATERIALS AND METHODS

3.1 RESEARCH DESIGN

The study adopted a cross-sectional design which involved 182 patients sampled from three hospitals who had conditions that were clinical risk factors for CKD (Diabetes and Hypertension)

Hypertension was defined by self-reported use of antihypertensive medications or an average of the second and third seated blood pressure measures: systolic 140 mm Hg or higher or diastolic 90 mm Hg or higher. Diabetes was defined as having a fasting blood glucose level of 7.0 mmol/l or higher, or a non-fasting blood glucose concentration of 11 mmol/l or higher or the use of insulin or oral hypoglycemic agents. Hypertensive and diabetics who were being managed were chosen for this study

3.2 STUDY SITE

The study was carried out at the Koforidua Regional Hospital, Enyeresi and Kibi Government Hospitals.

Koforidua Regional Hospital is the largest secondary point of referral for patients in the Eastern Region. Koforidua is a city and the capital of the Eastern Region in south Ghana. It is cosmopolitan in nature with an estimated population of 127,334. Koforidua has an annual rainfall ranging from 50 – 120 inches and 20 – 32 Celsius mean annual temperature

Kibi Hospital is in the East Akim Municipal District of the Eastern Region of Ghana. Kibi is a town and the capital of the East Akim District. The area receives between 1250mm to 1750

mm mean amount of rain each year. It has a settlement population of 11,677 people who are predominantly involved in agriculture.

Enyeresi Government Hospital in the Atiwa District of the Eastern region of Ghana. The District covers a total of 2950 square kilometers. It has an annual rainfall of 1200mm to 1500mm.Temperature ranges from a minimum of 26°C to a maximum of 30°C.Most of the people in the district are involved in farming activities. These Hospitals had the probability of providing experimental materials and/or patients for the study.

3.3 POPULATION AND SAMPLE SIZE

The target population of this study were patients who had been diagnosed with diabetes (Type II) and/or Hypertension in the various hospitals in the Eastern Region of Ghana. However, due to time, financial and other constraints, a sub-sample of this population was used. In all, a total of 182 patients were sampled for the study with the allowable margin of error been 5.0%. Of the 182 patients, 82 were sampled from the Koforidua Regional Hospital whilst 50 each were sampled from Kibi Government Hospital and Enyeresi Government Hospital respectively.

3.4 SAMPLING TECHNIQUE

Simple random stratified sampling technique was employed by the researcher in selecting the respondents at the various Government Hospitals for the study.

3.5 CONSENT PROCESS AND DOCUMENTATION

Ethical Clearance was obtained from the Committee on Human Research, Publication and

Ethics (CHRPE) of the School of Medical Sciences (SMS), Kwame Nkrumah University of Science and Technology (KNUST), Koforidua Regional Hospital, Kibi and Enyeresi Government Hospitals.

The consent of all research participants were obtained after adequate information had been presented to enable patients to voluntarily decide whether or not to participate in the study. Consent forms were given out to all research subjects for signing before the commencement of the study. However, options were made for opting out as a research participant when one wished.

3.6 ELIGIBILITY CRITERIA

Participants used for the study were carefully selected after fulfilling some specified criteria. Those selected were Patients who had:

- Been diagnosed of having Hypertension
- Been diagnosed of having Diabetes
- A complete medical record
- A follow up record from the time they were diagnosed with the conditions.

3.7 EXCLUSION CRITERIA

● HIV positive Diabetics and Hypertensive subjects.

● Study participants above 80 years and below 18 years

3.8 DATA COLLECTION AND DATA ANALYSIS

Survey questionnaires and interviews were used as the secondary research instruments for data collection. Consented participants (patients) were served with questionnaires to gather

information on their demographics, their health issues especially, with respect to kidney disease. Age, race, sex, were determined by self-report during the interviews. The questionnaires were first pre-tested using 50 Patients from the Koforidua Regional Hospital to ascertain the quality and state of the questions posed before distributing them to cover the entire samples (182) used. Out of the 182 questionnaires delivered, all 182 (100%) were received, analyzed and interpreted by the researcher.

Data collected from the survey questionnaires delivered were analyzed using the Statistical Package for Social Sciences (SPSS) software version 19.0 and Excel 2010. To make valid analysis on the presentation and analysis of the data collected from the field, the researcher used inferential statistics in addition to descriptive statistics such as tables and frequency counts and charts to depict the relevant data.

3.9 BLOOD SAMPLE COLLECTION

After the collection of baseline participant information through the survey interview, trained technicians then collected specimen. Eight (6) mls of blood sample was collected from participants. After centrifugation (500 revolutions for 10 min), samples of serum were aliqoted into two sample tubes and stored at −20 °C prior to analyses.

3.10 BIOCHEMICAL ANALYSIS

The BT 3000 Chemistry analyzer (Elan Diagnostics, Smithfield, CA, USA) was used for all biochemical analyses. JAZ reagents were used.

3.10.1 Albumin (ALB)

The method used for this assay is based on that of Doumas et al., (1971) in which at a controlled pH, bromocresol green (BCG) forms a coloured complex with albumin. The intensity of the colour at 630 nm is directly proportional to the albumin content

$$BCG + Albumin \xrightarrow{controlled\ pH} Green\ BCG{-}Albumin\ Complex$$

3.10.2 Total protein (TP)

The present method is based on the modifications of Gornall et al., (1949). Protein in serum forms a blue coloured complex when reacted with cupric ions in an alkaline solution. The intensity of the violet colour is proportional to the amount of proteins present when compared to a solution with known protein concentration. The wavelength used was 540nm.

$$Protein + Cu^{2+}\ alkali \longrightarrow Coloured\ complex$$

3.10.3 Blood Urea Nitrogen (BUN)

Determination of urea nitrogen in serum is widely used as a screening test for renal determination of creatinine function. When used in conjunction with the in serum, it is helpful in the differential diagnosis of the three types of azotemia; pre-renal, renal and postrenal.

The present procedure is based on a modification of the method of Talke and Schubert (1965). Urea is hydrolyzed in the presence of water and urease to produce ammonia and carbon dioxide. The liberated ammonia reacts with α-ketoglutarate in the presence of NADH and Glutamate dehydrogenase to form L- Glutamate and NAD+. As the reaction proceeds, the absorbance at 340nm decrease. The initial rate of this change is proportional to the amount of urea in the sample.

$$Urea + H_2O \xrightarrow{UREASE} 2NH_3 + CO_2$$

$$NH_3 + \alpha\text{-ketoglutarate} + NADH \xrightarrow{GLDH} L\ Glutamate + NAD$$

3.10.4 Creatinine

Creatinine measurements are used in the assessment of renal dysfunction. Elevated creatinine levels are found in renal diseases and insufficiency with decreased glomerular filtration (uremia or azotemia if severe); urinary tract obstruction; reduced renal blood flow including congestive heart failure, shock and dehydration. This method is based on a modification of the kinetic procedure which is fast, simple and avoids interferences (Fabiny and Ertingshausen, 1971), incorporating a surfactant and other reagents to minimize protein and carbohydrate interferences. Creatinine reacts with picric acid in alkaline conditions to form a colour complex (yellow-orange) which absorbs at 510 nm. The rate of formation of colour is proportional to the creatinine in the sample

$$Creatinine + Sodium\ Picrate \xrightarrow{alkali} Creatinine - picrate\ complex$$

3.10.5 Uric Acid

Uric acid is converted by oxidation by uricase to allantoin and H_2O_2, which under the catalytic influence of peroxidase, oxidizes 3, 5-dichloro-2-hydroxybenzene- sulphonic acid (chlorophenol sulphonic acid) and 4-aminophenazone (4AP) to form a red-violet quinonimine compound, which is proportional to the amount of uric acid present

$$Uric\ acid + O^2 + 2H_2O_2 \xrightarrow{uricase} Allantoin + CO_2 + H_2O_2$$

$$2H_2O_2 + chlorophenol\ sulphonic\ acid + 4AP \xrightarrow{peroxidase}$$

$$N-4-antipyryl-3-chloro-5-sulphonate-p-benzo-quinoneimine + H_2O$$

3.11 ANALYSIS OF PLASMA PROTEINS

3.11.1 Beta 2 Microglobulin

Principle and Method

Beta-2-Microglobulin is an indirect solid phase enzyme immunometric assay (ELISA). The micro plate from RnD Systems had been coated with much purified anti-h-Beta-2Microglobulin antibodies. The plate can be divided into 12 parts of 8 wells each or can be used completely for 96 determinations. Each well can be separated from the module ("breakaway"). The binding of the analyte, the formation of the sandwich complexes and the enzymatic color reaction take place during three different reaction phases: Phase 1: 100ul of calibrators, controls and prediluted patient samples were pipetted into the wells of the microplate. Any present Beta-2-Microglobulin bind to the immobilized anti-Beta-2Microglobulin antibodies. After a 30 minutes incubation the micro plate was washed with wash solution to remove non-reactive serum components. Phase 2: 100ul of anti-h-Beta-2Microglobulin-horseradish peroxidase conjugate solution was pipetted into the wells to recognize Beta-2-Microglobulin molecules bound to the immobilized antibodies by forming the sandwich complexes. After 15 minutes incubation any excessive enzyme conjugate, which was not specifically bound was washed away with wash solution. Phase 3: A chromogenic substrate solution containing TMB (3, 3`, 5, 5`-Teramethyl-benzidine) was dispensed into the wells. During 15 minutes of incubation the color of the solutions changed into blue. Color development was stopped by adding 1 M hydrochloric acid as stop solution. The solutions color changed into yellow. The amount of colour is directly proportional to the concentration of Beta-2-Microglobulin present in the original sample. This was read at

450nm

3.11.2 Cystatin C

Principle and Method

This assay employed the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human Cystatin C had been pre-coated onto a microplate from RnD systems. 100ul of Standards and samples were pipetted into the wells and any Cystatin C present is bound by the immobilized antibody. After washing away any unbound substances, 100ul of enzyme-linked monoclonal antibody specific for human Cystatin C was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, 90ul of substrate solution was added to the wells and color developed in proportion to the amount of Cystatin C bound in the initial step. The color development was stopped and the intensity of the color was measured at 450nm.

3.12 ASSESSMENT OF CKD

The calculated eGFR was used to stratify the study population into stages of CKD, based on the staging system of the Kidney Disease: Outcome Quality Initiative. (KDOQI) guidelines for CKD. The various stages were defined as follows: Stage 1 (Kidney damage with normal or increased eGFR) = ≥90 ml/min/1.73 m^2; Stage 2 (Kidney damage with mildly decreased eGFR) = 60–89 ml/min/ 1.73 m^2; Stage 3a (mild to moderately decreased eGFR) = 45–59 ml/min/1.73 $m^{2;}$ Stage 3b (moderate to severely decreased eGFR) = 30–44 ml/min/1.73 $m^{2;}$ Stage 4 (severely decreased eGFR) = 15–29 ml/min/1.73 m2 and Stage 5 (Kidney failure).

3.13 STATISTICAL ANALYSIS

Statistical analyses were performed using Graph Pad Prism (www.graphpad.com). Data were expressed as mean values and 95% confidence intervals (CIs) unless indicated otherwise. Inverses of Cr, cystatin C, and B2M were correlated with MDRD and the inverses of creatinine, cystatin C, and B2M were used to obtain a direct correlation with MDRD. Correlations between creatinine, cystatin C, and B2M were as well performed. The diagnostic value of cystatin C and B2M were evaluated using receiver operating characteristic (ROC) curve analysis. Sensitivity, specificity, and positive likelihood ratios were calculated. Correlations between quantitative data were determined using Pearson's test. A *p* value of less than or equal 0.05 was considered statistically significant.

3.14 BIOSAFETY AND QUALITY CONTROL

The study was conducted in accordance with the Ethical Principles of the CHRPE, KNUST/SMS and under standard laboratory practices. All wastes were disposed of in accordance with the waste management protocol. The study was subjected to a high quality external quality control (EQC) checks and about 10% of cases were randomly selected and delivered to Kumasi Centre for Collaborative Research for validation of results.

CHAPTER 4

RESULTS

Table 4.1 shows age, clinical conditions and biochemical profile stratified by gender. Out of 182 participants, the most represented age group was 60-79 years [45.1% (82/182)]. The mean

age of the general study participants was 57.59 ± 0.94 years. No statistically significant difference was observed between the mean age of males compared to females (p=0.4513). Higher proportion (54.4%) of the participants had both diabetes and hypertension (DM+HTN) followed by hypertension (HTN) only (30.2%) and only diabetes (DM) (15.4%). There was a significantly elevated mean levels of Cr, uric acid, cystatin C and B2M in males compared to females (p<0.05). Means levels of urea, total protein, albumin, GFR both criteria, EPI Cys C and EPI-Cr-Cys C in male participants were not statistically significant difference compared to females (p>0.05).

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Table 4.1 Age, clinical characteristics and biochemical profile of participants categorised by gender

Parameters	Total (n=182)	Males (n=42)	Females (n=140)	p-value
Age (years)	57.59 ± 0.9372	58.88 ± 1.72	57.20 ± 1.11	0.4513

Age Group				0.5462
20-39	17	2	15	
40-59	81	19	62	
60-79	82	21	61	
≥80	2	0	2	
Conditions				0.9471
DM	28	7	21	
HTN	55	13	42	
DM + HTN	99	22	77	
Biochemical profile				
Cr (µmol/l)	92.24 ± 2.48	109.0 ± 6.56	87.20 ± 2.42	**0.0002**
Urea (mmol/l)	5.02 ± 0.38	5.75 ± 0.79	4.797 ± 0.43	0.2931
Total protein (g/L)	92.25 ± 0.81	92.67 ± 1.49	92.12 ± 0.97	0.7801
Albumin (g/L)	45.41 ± 0.34	45.67 ± 0.44	45.33 ± 0.43	0.6788
Uric acid (mmol/l)	502.8 ± 16.04	576.4 ± 39.60	480.7 ± 16.78	**0.0116**
Cystatin C (mg/ml)	0.99 ± 0.03	1.15 ± 0.09	0.95 ± 0.04	**0.0223**
B2M (mg/ml)	2.27 ± 0.10	2.68 ± 0.29	2.146 ± 0.11	**0.0394**
eGFR-MDRD	84.99 ± 2.20	86.15 ± 3.35	84.63 ± 2.69	0.7724
eGFR-EPI	82.09 ± 2.11	82.05 ± 3.66	82.10 ± 2.51	0.9917
EPI-Cr-Cys C	82.94 ± 2.10	81.00 ± 4.21	83.52 ± 2.43	0.6144

Values are presented as mean ± standard error of mean (SEM). Un-paired t-test was used to compare means (Males vs Females). p<0.05 was considered as statistically significance.

Table 4.2 summarises the biochemical profiles of participants with only DM, only HTN and both DM+HTN. There was a statistically significant difference between the means age, total protein, uric acid, eGFR (both criteria), and EPI-Cr-Cys C levels among participants with DM,

HTN and both DM + HTN ($p<0.0001$). Participants with both DM+HTN and only HTN were significantly older than those with only DM ($p<0.0001$). Participants with both DM + HTN had a significantly lower total protein compared to those presenting with only HTN ($p<0.0001$). Serum Uric acid levels were significantly elevated among participant with both DM+HTN compared to those with only HTN ($p<0.0001$). Estimated GFR was significantly ($p<0.05$) elevated among participants presenting with only DM compared to both DM+HTN and only HTN. Study participants presenting with only DM had a significantly elevated EPI-Cr-Cys C levels compared to those with both DM+HTN ($p=0.0295$).

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Table 4.2. Age and Biochemical profiles stratified by diabetes, hypertension and both diabetes and hypertension

Parameters	DM (n=28)	HTN (n=55)	DM + HTN (n=99)	P-value	Significant pairs
Age (years)	47.32 ± 3.01	59.95 ± 1.55***	59.18 ± 1.09***	**< 0.0001**	DM vs HTN, DM vs DM+HTN
Cr (mmol/l)	85.64 ± 5.56	90.36 ± 2.47	95.15 ± 4.06	0.3691	None
Urea (mmol/l)	4.507 ± 0.36	5.605 ± 1.06	4.835 ± 0.37	0.5751	None
Total protein (g/L)	93.32 ± 2.01	97.55 ± 1.51	89.00 ± 0.99***	**< 0.0001**	HTN vs. DM+HTN
Albumin (g/L)	44.89 ± 0.74	45.42 ± 0.45	45.55 ± 0.54	0.8061	None
Uric acid	482.1 ± 27.36	406.7 ± 19.61	562.0 ± 24.66***	**< 0.0001**	HTN vs. DM+HTN
Cystatin C	0.90 ± 0.07	0.92 ± 0.04	1.06 ± 0.06	0.12	None
B2Microglobulin	2.13 ± 0.19	1.972 ± 0.09	2.475 ± 0.19	0.1111	None
eGFR-MDRD	98.67 ± 7.85	81.23 ± 3.12*	83.20 ± 2.84*	**0.0269**	DM vs. HTN, DM vs. DM+HTN
eGFR-EPI	98.14 ± 7.81	78.11 ± 2.72**	79.76 ± 2.67**	**0.0043**	DM vs. HTN, DM vs. DM+HTN
EPI-Cr-Cys C	95.93 ± 6.36	81.13 ± 2.93	80.27 ± 2.94*	**0.0295**	DM vs. DM+HTN

Values are presented as mean ± standard error of mean (SEM). One-way ANOVA followed by Tukey Post Hoc multiple comparism was used to compare means (DM vs HTN vs DM+HTN) p<0.05 was considered as statistically significance. *p<0.01; **p<0.001; ***p<0.0001.

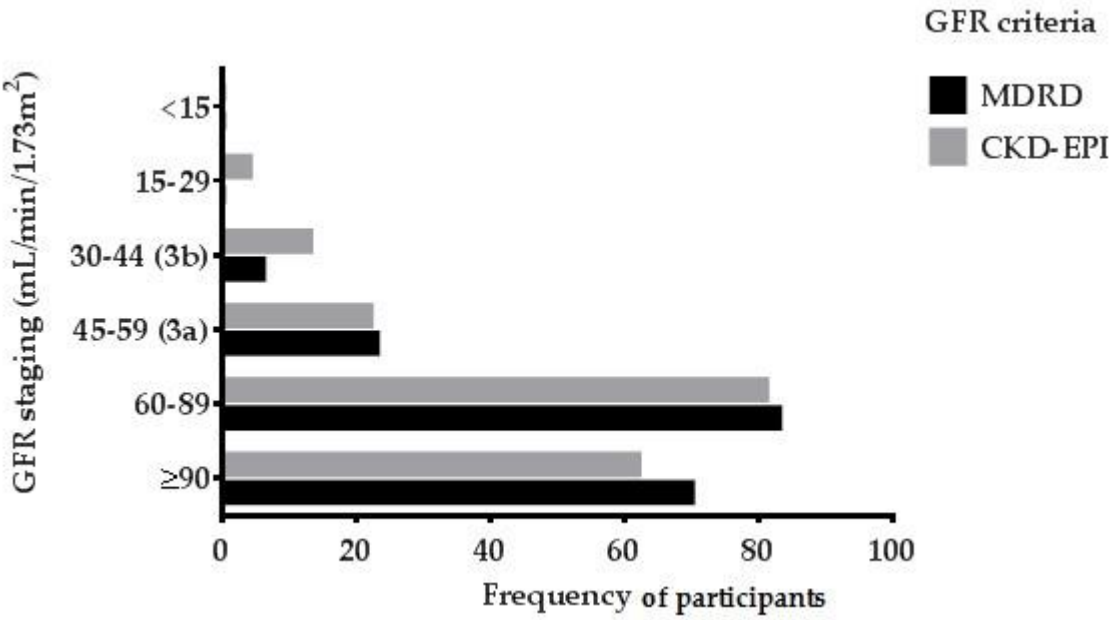

Figure 4.1 GFR staging categorised by MDRD and CKD-EPI criteria

As shown in **Figure 4.1**, the number of participants with CKD stage 1, 2 and 3a were higher using the MDRD criteria compared to CKD-EPI. Meanwhile, a higher number of participants had CKD stage 3b and 4 using CKD-EPI equation compared to staging using the MDRD equation.

Figure 4.2 showed that higher a proportion of females than male participants had CKD stage 1-4. There was however no significant association between GFR and gender irrespective of the GFR equation used ($p>0.05$).

In **Table 4.3** higher proportion of the older age group (60-79 years) had GFR stage 3a and 3b when MDRD equation was used and GFR stage 3a, 3b and 4 when CKD-EPI was used compared to the younger age group. There was a significant association between GFR and age ($p<0.0001$).

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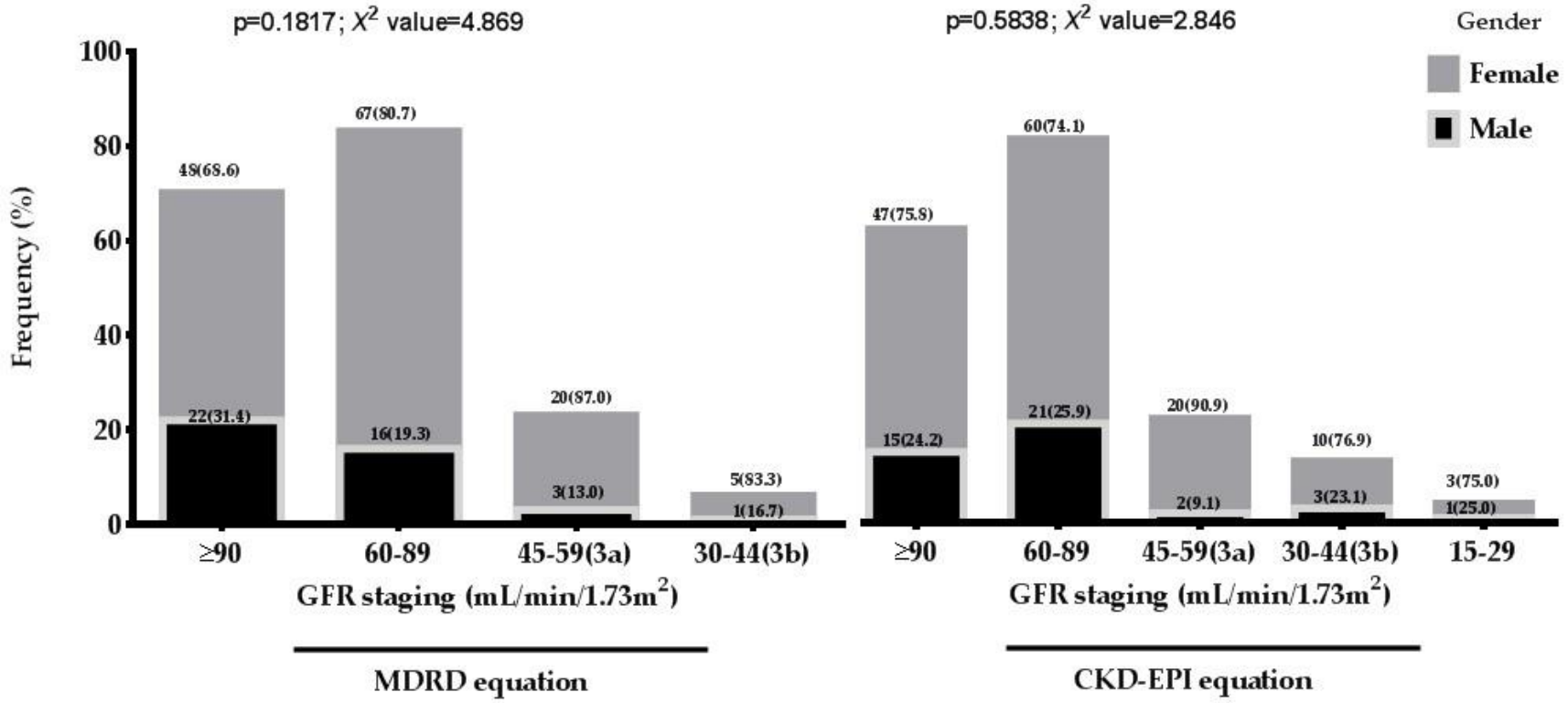

Figure 4.2 Association between GFR and Gender using the MDRD and CKD-EPI equation

Table 4.3 Association between age group and GFR using MDRD and CKD-EPI equations

GFR staging (ml/min/1.73 m2)	Total	Age Group				X^2 value; p-value
		20-39 (n=17)	40-59 (n=81)	60-79 (n=82)	≥80 (n=2)	
MDRD equation						
≥90	70	14(20.0)	36(51.4)	20(28.5)	-	38.47 ; < 0.0001
60-89	83	3(3.6)	36(43.4)	43(51.8)	1(1.2)	
45-59 (3a)	23	-	8(34.8)	15(65.2)	-	
30-44 (3b)	6	-	1(16.7)	4(66.7)	1(16.7)	
15-29	-	-	-	-	-	
<15	-	-	-	-	-	
CKD-EPI equation						
≥90	62	15(24.2)	35(56.5)	12(19.4)	-	49.81 ; < 0.0001
60-89	81	2(2.4)	37(45.7)	41(50.6)	1(1.2)	
45-59 (3a)	22	-	8(36.4)	14(63.6)	-	
30-44 (3b)	13	-	1(7.6)	11(46.2)	1(7.6)	
15-29	4	-	-	4(100.0)	-	
<15	-	-	-	-	-	

Values are presented as frequency (%). Chi-square (X^2) was used to test association between proportions. P<0.05 was considered as statistically significant

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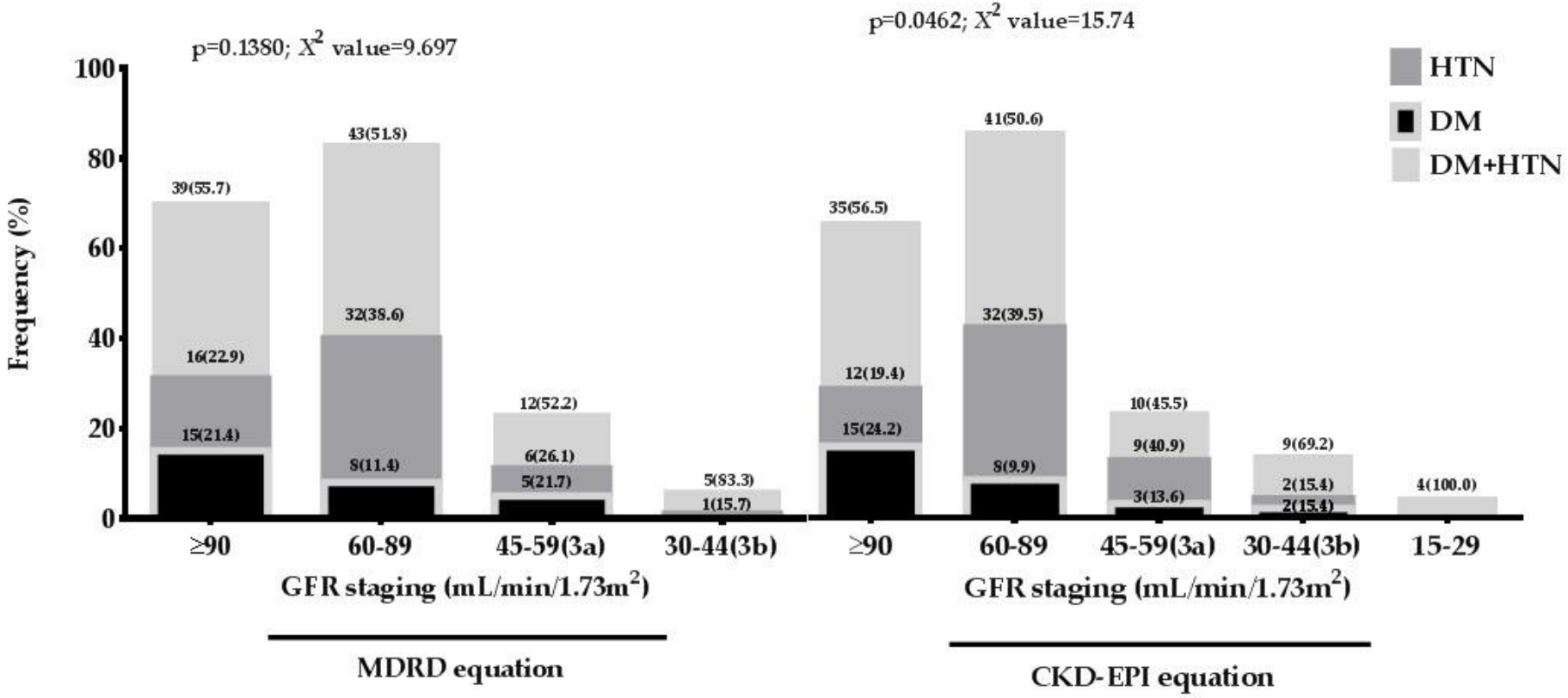

Figure 4.3 Association between GFR and DM, HTN, and DM+HTN condition using the MDRD and CKD-EPI equation

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As shown in **Figure 4.3** higher proportion of the participants with both DM+HTN had CKD (GFR stage 3a 52.2%(12/23) and 3b 83.3%(5/6) using MDRD equation and GFR stage 3a 45.5%(10/22), 3b 69.2%(9/13) and stage 4, 100%(1/1) using CKD-EPI equation) followed by those with HTN (GFR stage 3a 26.1%(6/23) and 3b 15.7(1/6) using MRDR equation and GFR stage 3a 40.9%(9/22), and 3b 15.4%(2/13) using CKD-EPI equation and DM (GFR stage 3a 21.7%(5/23) using MRDR equation and GFR stage 3a 13.6%(3/22), and 3b 15.4%(2/13) using CKD-EPI equation). There was a significant association between GFR and DM, HTN and both DM+HTN using CKD-EPI equation

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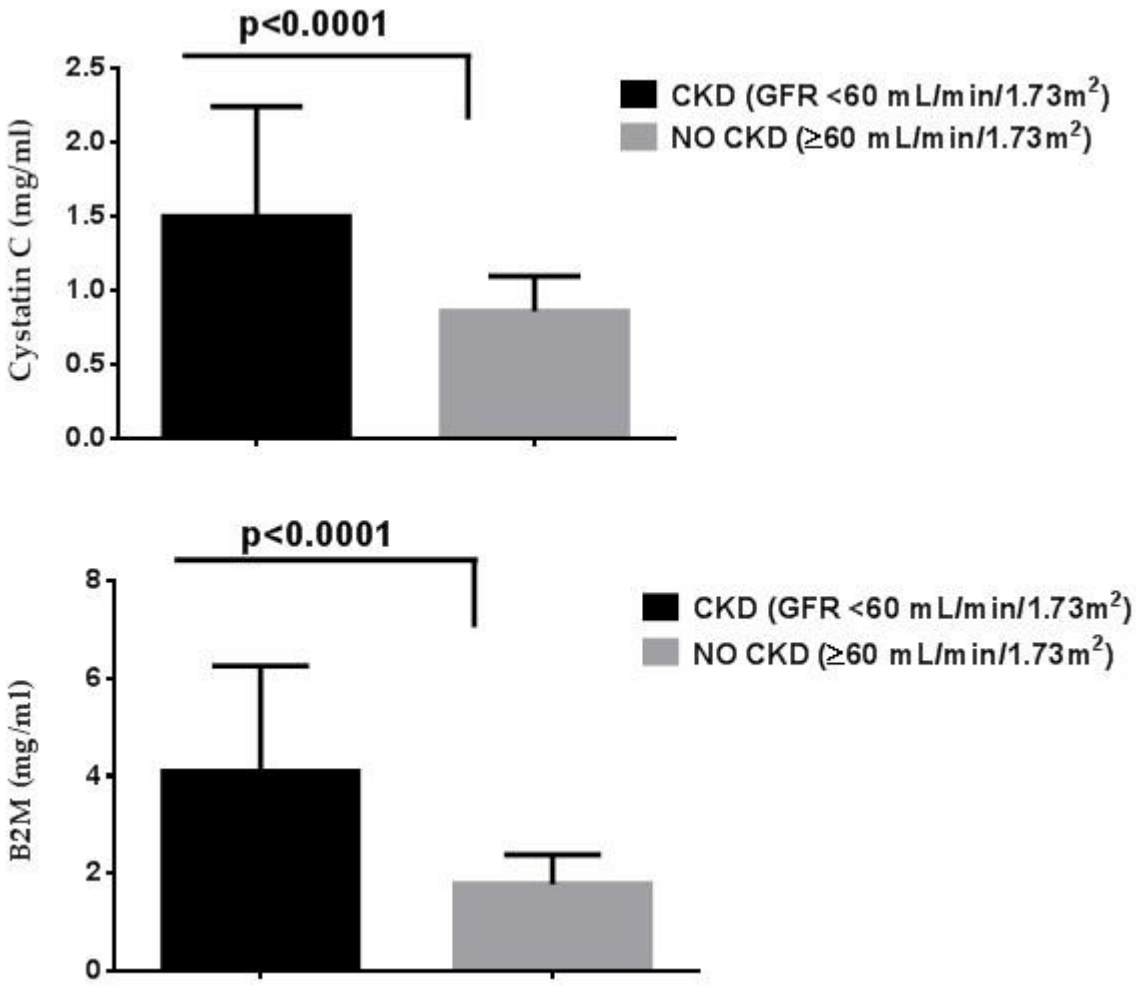

Figure 4.4 Levels of Cystatin C and B2 Microglobulin (B2M) among the general participants (DM, HTN and DM+HTN) with CKD and without CKD according to CKDEPI equation

As shown in **Figure 4.4,** Mean levels of Cystatin C and B2M were significantly elevated among participants with CKD compared to those without CKD (p<0.0001).

In **Table 4.4,** general study participants with CKD were significantly older than those without CKD ($p<0.0001$). Participants with CKD had a significantly elevated mean levels of Cr and uric acid and compared to those without CKD ($p<0.001$). Mean levels of urea, total protein and albumin were elevated among participants with CKD compared to those without CKD ($p>0.05$). Meanwhile, EPI-Cr-Cys C, cystatin C based equations were significantly lower among participants with CKD compared to those without CKD ($p<0.0001$).

Table 4.4. Biochemical profile and cystatin C equations among the general participants with CKD and without CKD according to CKD-EPI equation

	GFR<60mL/min/1.73m^2	GFR≥60mL/min/1.73m^2	
Parameters	**CKD** (n=39)	**NO CKD** (n=143)	p-value
Age (years)	64.62 ± 1.46	55.67 ± 1.07	< 0.0001
Cr (mmol/l)	136.6 ± 6.74	80.15 ± 1.38	< 0.0001
Urea (mmol/l)	6.043 ± 0.42	4.738 ± 0.47	0.1467
Total protein (g/L)	93.97 ± 2.39	91.78 ± 0.82	0.2722
Albumin (g/L)	46.23 ± 1.27	45.18 ± 0.27	0.2100
Uric acid (mmol/l)	649.4 ± 38.53	462.8 ± 16.02	< 0.0001
EPI-Cr-Cys C	50.82 ± 3.24	91.70 ± 1.97	< 0.0001

Values are presented as mean ± standard error of mean (SEM). P< 0.05 was considered as statistically significant.

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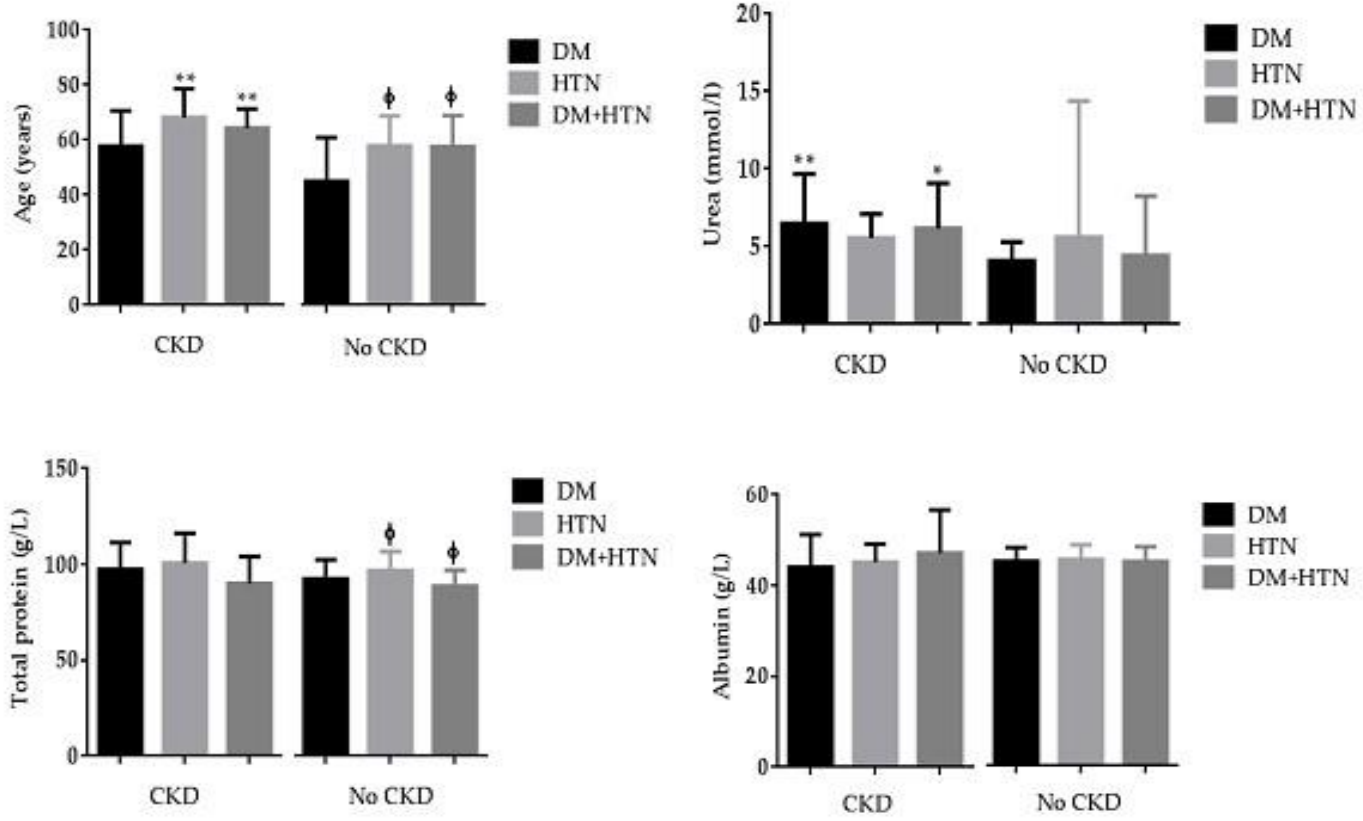

Figure 4.5 Mean age, levels of urea, total protein, and albumin among DM, HTN and DM+HTN participants with and without CKD.

****p<0.01; **p<0.001. Bars with * indicate significant difference compared to corresponding condition with no CKD. Bars with ϕ indicates significant pairs within each group.***

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As shown in **Figure 4.5,** CKD participants with HTN, and both DM+HTN were significantly older compared to their corresponding participants without CKD ($p<0.001$). CKD participants with DM and both DM+HTN had significantly elevated mean serum levels of urea compared to their corresponding participants without CKD ($p<0.05$). Within the group without CKD, participant with HTN, and both DM+HTN were significantly older compared to those with only DM ($p<0.05$). Participants with HTN had a significantly elevated mean total protein compared to those with both DM+HTN within the group without CKD condition ($p<0.05$).

In Figure 4.6, CKD participants with DM, HTN and both DM+HTN had significantly elevated levels of cystatin C and B2M and a reduced level of EPI-Cr-Cys c compared to their corresponding participants without CKD ($p<0.0001$). CKD participants with both DM+HTN had a significantly elevated mean levels of uric acid compared to their corresponding participants without CKD ($p<0.0001$). Within the group without CKD, participants with both DM+HTN had significantly elevated mean levels of uric acid compared to those with HTN ($p<0.05$). Again, participants with DM had a significantly elevated levels of EPI-Cr-Cys c compared to those with HTN within the group without CKD condition ($p<0.05$).

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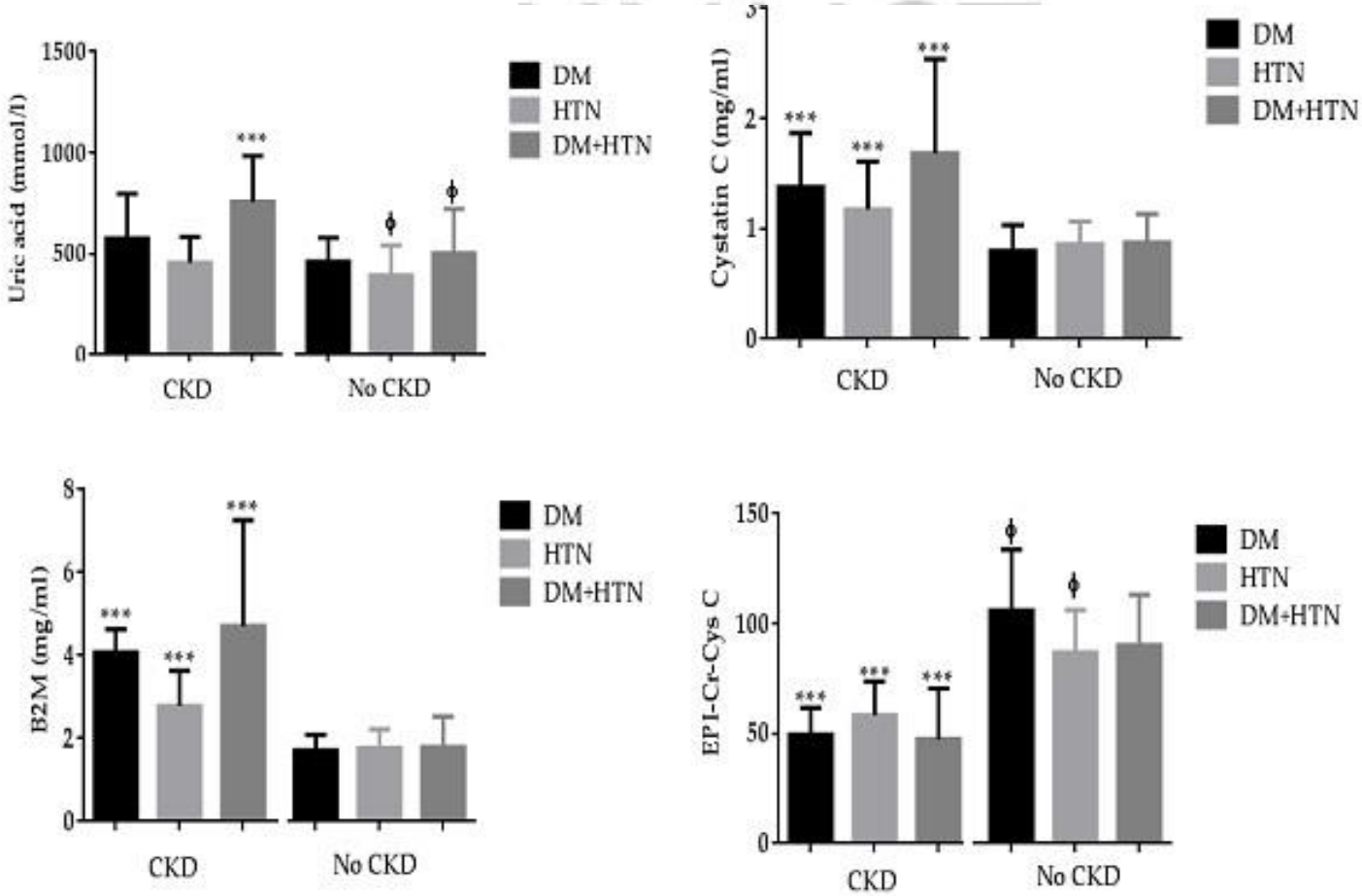
Figure 4.6 *Mean levels of uric acid, cystatin c, B2M and EPI-Cr-Cys C among DM, HTN and DM+HTN participants with and without*



*CKD*p<0.01; **p<0.001; ***p<0.0001. Bars with * indicate significant difference compared to corresponding condition with no CKD. Bars with ϕ indicates significant pairs within each group.*

Table 4.5 Mean age and biochemical profile of DM, HTN and DM+HTN participants with and without CKD

	CKD				NO CKD			
Parameters	DM (n=5)	HTN (n=11)	DM+HTN (n=23)	p-value	DM (n=23)	HTN (n=44)	DM+HTN (n=76)	p-value
Age (years)	57.80 ± 5.79	68.27 ± 3.12**	64.35 ± 1.44**	0.1000	45.04 ± 3.30$_b$	57.86 ± 1.642$_b$	57.62 ± 1.30a	< 0.0001
Cr (mmol/l)	134.4 ± 8.68***	111.6 ± 3.64$_b$***	149.0 ± 10.33$_b$***	0.0489	75.04 ± 3.83$_b$	85.05 ± 2.36b	78.87 ± 1.82	0.0365
Urea (mmol/l)	6.48 ± 1.43**	5.563 ± 0.47	6.17 ± 0.61*	0.7576	4.07 ± 0.26	5.616 ± 1.32	4.429 ± 0.44	0.4483
Total protein (g/L)	97.40 ± 6.27	100.6 ± 4.73	90.04 ± 2.92	0.1313	92.43 ± 2.07	96.77 ± 1.49$_b$	88.68 ± 0.96$_b$	< 0.0001
Albumin (g/L)	44.00 ± 3.24	45.18 ± 1.18	47.22 ± 1.98	0.6369	45.09 ± 0.62	45.48 ± 0.49	45.04 ± 0.37	0.7611
Uric acid(mmol/l)	577.0 ± 99.02	456.1 ± 38.76$_b$	757.6 ± 47.30$_b$***	0.0009	461.5 ± 24.95	394.3 ± 22.33$_b$	502.8 ± 25.21$_b$	0.0105
Cystatin C (mg/ml)	1.38 ± 0.22***	1.17 ± 0.13***	1.683 ± 0.18***	0.1626	0.79 ± 0.05	0.86 ± 0.03	0.87 ± 0.03	0.4026
B2M (mg/ml)	4.07 ± 0.25***	2.78 ± 0.26b***	4.714 ± 0.53b***	0.0483	1.71 ± 0.08	1.76 ± 0.07	1.79 ± 0.08	0.823
EPI-Cr-Cys C	49.60 ± 5.46***	58.36 ± 4.63***	47.48 ± 4.81***	0.3454	106.0 ± 5.79$_b$	86.82 ± 2.92$_b$	90.20 ± 2.64$_b$	0.0041

Values are presented as mean ± standard error of mean (SEM). P< 0.05 was considered as statistically significant. (*p<0.01; **p<0.001; ***p<0.0001. indicates significance in comparison with same participants without CKD. Values with same subscript ($_b$) are significant pairs within each CKD and No CKD group of DM, HTN and DM+HTN.

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Table 4.6 shows the bivariate and partial Pearson's correlation between GFR and biochemical profile. There was a significant negative correlation (r= -0.947; p=0.004) between CKD and age among participants with DM. Significant negative correlation was observed between CKD and urea (r= -0.624; p=0.04), Total protein (r= -0.692; p=0.018), uric acid (r= -0.792; p=0.004) and Beta 2 Microglobulin (r= -0.743; p=0.009) among participants with HTN. There was a significant negative correlation between CKD and age (r= -0.525; p=0.010), urea (r= -0.786; p<0.0001), uric acid (r= -0.464; p=0.026), cystatin C (r= -0.761; p<0.0001) and Beta 2 Microglobulin (r= -0.901; p<0.0001) among participants with both DM+HTN.

Among diabetic (DM) participants without CKD, GFR correlated negatively with age (r= 0.705; p=0.0002), and cystatin C (r= -0.587; p=0.003). Among hypertensive (HTN) participants without CKD, GFR correlated negatively with age (r= -0.337; p=0.025), total protein (r= -0.361; p=0.016), cystatin C (r= -0.339; p=0.025) and Beta 2 Microglobulin (r= 0.429; p=0.004). GFR negatively correlated with age (r= -0.505; p<0.0001), urea (r= -0.264; p=0.0214), uric acid (r= -0.264; p=0.021), cystatin C (r= -0.520; p= <0.0001) and Beta 2 Microglobulin (p= -0.500; p<0.0001).

Analysis on partial Pearson correlation after adjusting for age showed that all significant correlation holds except for urea vs CKD in HTN, uric acid vs CKD in both DM+HTN, urea vs No CKD in DM, cystatin C vs No CKD in DM, and cystatin C vs No CKD in HTN (p>0.05).

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Table 4.6. Bivariate and Partial Pearson correlation of age and biochemical profile of DM, HTN and DM+HTN participants with and without CKD

	CKD (GFR<60mL/min/1.73m2)			No CKD(GFR≥60mL/min/1.73m2)		
	DM	HTN	DM+HTN	DM	HTN	DM+HTN
Parameters	r ; p-value	r; p-value	r; p-value	r; p-value	r; p-value	r; p-value
Age	-0.947; 0.004	-0.468; 0.147	-0.525; 0.010	-0.705; 0.0002	-0.337; 0.025	-0.505 ; <0.0001
Urea	-0.717; 0.173	-0.624; 0.04	-0.786; <0.0001	-0.266; 0.220	-0.026; 0.865	-0.264 ; 0.0214
Partial cor.	***-0.697; 0.303***	***-0.555; 0.096***	***-0.797; <0.0001***	***-0.359; 0.100***	***-0.026; 0.870***	***-0.260; 0.024***
Total protein	-0.099; 0.875	-0.692; 0.018	-0.091; 0.681	0.017; 0.938	-0.361; 0.016	-0.140 ; 0.229
Partial cor.	***0.912; 0.088***	***-0.719; 0.019***	***-0.101; 0.655***	***-0.206; 0.357***	***-0.416; 0.006***	***-0.159; 0.172***
Albumin	0.197; 0.751	-0.188; 0.579	0.029; 0.895	-0.180; 0.411	-0.051; 0.741	-0.122 ; 0.296
Partial cor.	***0.029; 0.971***	***-0.313; 0.379***	***0.017; 0.941***	***-0.059; 0.795***	***0.002; 0.988***	***-0.179; 0.124***
Uric acid	-0.745; 0.149	-0.792; 0.004	-0.464; 0.026	-0.02; 0.928	-0.190; 0.216	-0.264 ; 0.021
Partial cor.	***0.234; 0.766***	***-0.825; 0.003***	***-0.302; 0.172***	***-0.287; 0.195***	***-0.284; 0.065***	***-0.247; 0.032***
Cystatin C	0.065; 0.918	-0.133; 0.696	-0.761; <0.0001	-0.587; 0.003	-0.339; 0.025	-0.520 ; <0.0001
Partial cor.	***0.370; 0.63***	***-0.110; 0.763***	***-0.715; <0.0001***	***-0.423; 0.050***	***-0.295; 0.054***	***-0.504; <0.0001***
B2Microglobulin	0.751; 0.144	-0.743; 0.009	-0.901; <0.0001	-0.228; 0.296	-0.429; 0.004	-0.500 ; <0.0001

Partial cor.	***-0.132; 0.868***	***-0.673; 0.033***	***-0.897; <0.0001***	***-0.298; 0.177***	***-0.404; 0.007***	***-0.484; <0.0001***

r: correlation coefficient. Values are presented as correlation coefficient; p-value. Values in boldface and italic are partial correlation. p<0.05 was considered statistically significant.

Table 4.7 shows the correlation of cystatin C and B2M with age and biochemical profile among participants with CKD. There was a significant negative correlation between B2M and uric acid among DM (r= -0.962; p=0.009) and HTN (r= -0.962; 0.009) participants respectively. Cystatin C correlated positively with age (r=0.374; p<0.0001), urea (r= 0.705; <0.0001) and total protein (r= 0.392; <0.0001) among participants with both DM+HTN. There was a significant positive correlation between B2M and age (r=0.358; <0.0001), urea (r=0.897; p<0.0001) and total protein (r= 0.109; p<0.0001). Table 4.7 Pearson correlation of cystatin C and B2M with age and biochemical profile of DM, HTN and DM+HTN participants with CKD

	DM with CKD		HTN with CKD		DM+HTN with CKD	
	Cys C	B2M	Cys C	B2M	Cys C	B2M
Age (years)	0.056; 0.928	-0.818; 0.09	0.056; 0.928	-0.818; 0.09	0.374; **<0.0001**	0.358; **<0.0001**
Cr (mmol/l)	0.527; 0.361	-0.524; 0.365	0.527; 0.361	-0.524; 0.365	0.722; 0.0786	0.937; 0.0931
Urea (mmol/l)	0.320; 0.599	-0.338; 0.578	0.32; 0.599	-0.338; 0.578	0.705; **<0.0001**	0.897; **<0.0001**
Total protein (g/L)	0.352; 0.561	-0.595; 0.29	0.352; 0.561	-0.595;0.290	0.392; **<0.0001**	0.109; **<0.0001**
Albumin (g/L)	0.303; 0.62	-0.289; 0.637	0.303; 0.62	-0.289; 0.637	-0.079; 0.0641	-0.019; 0.621

Uric acid (mmol/l)	0.409; 0.494	-0.962; **0.009**	0.409; 0.494	-0.962; **0.009**	0.480; 0.722	0.343; 0.933

r: correlation coefficient. Values are presented as correlation coefficient; p-value. p<0.05 was considered statistically significant

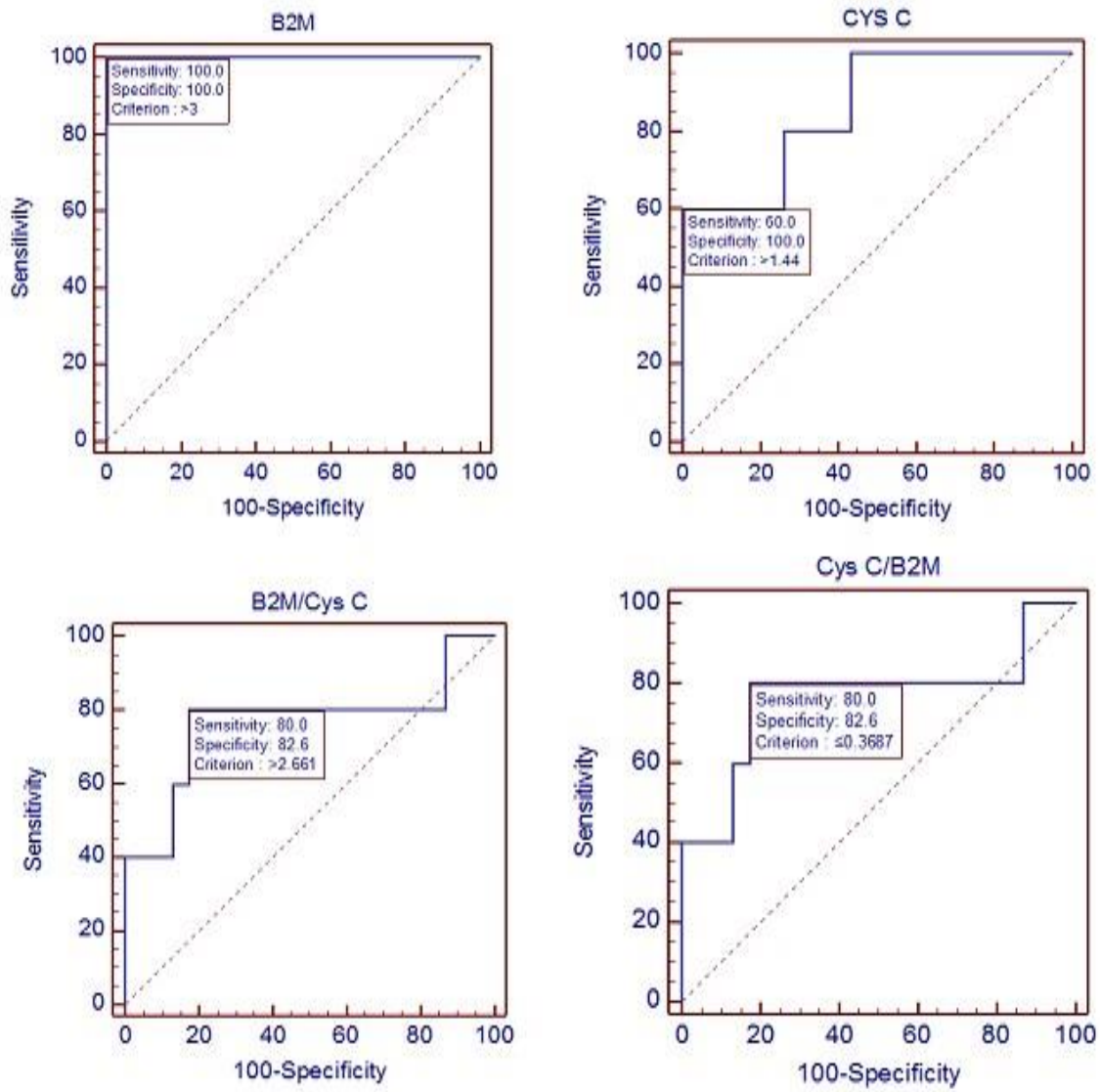

Figure 4.7 Receiver operating characteristic (ROC) curve of biomarkers in predicting CKD among participants with DM only

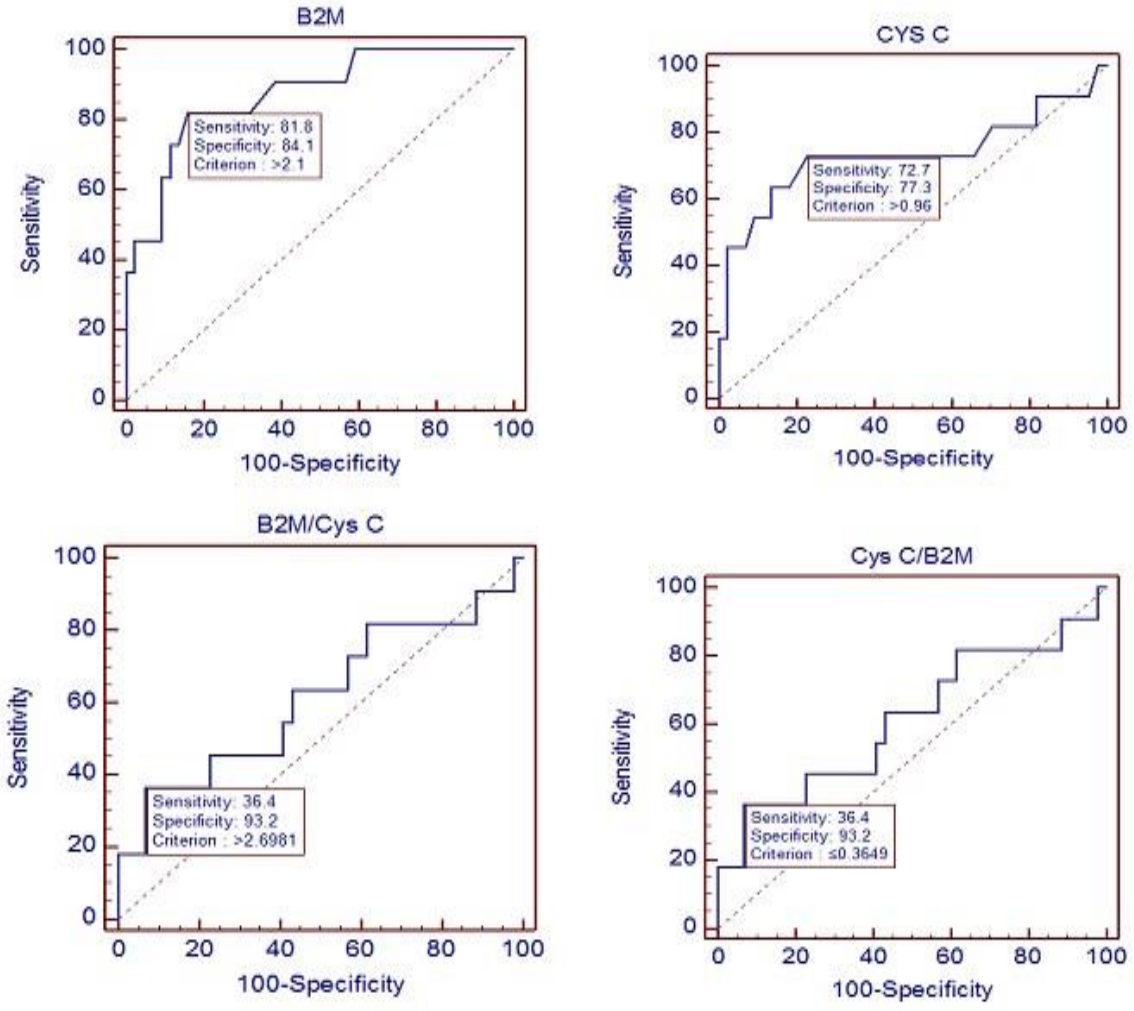

Figure 4.8 Receiver operating characteristic (ROC) curve of biomarkers in predicting CKD among participants with HTN only

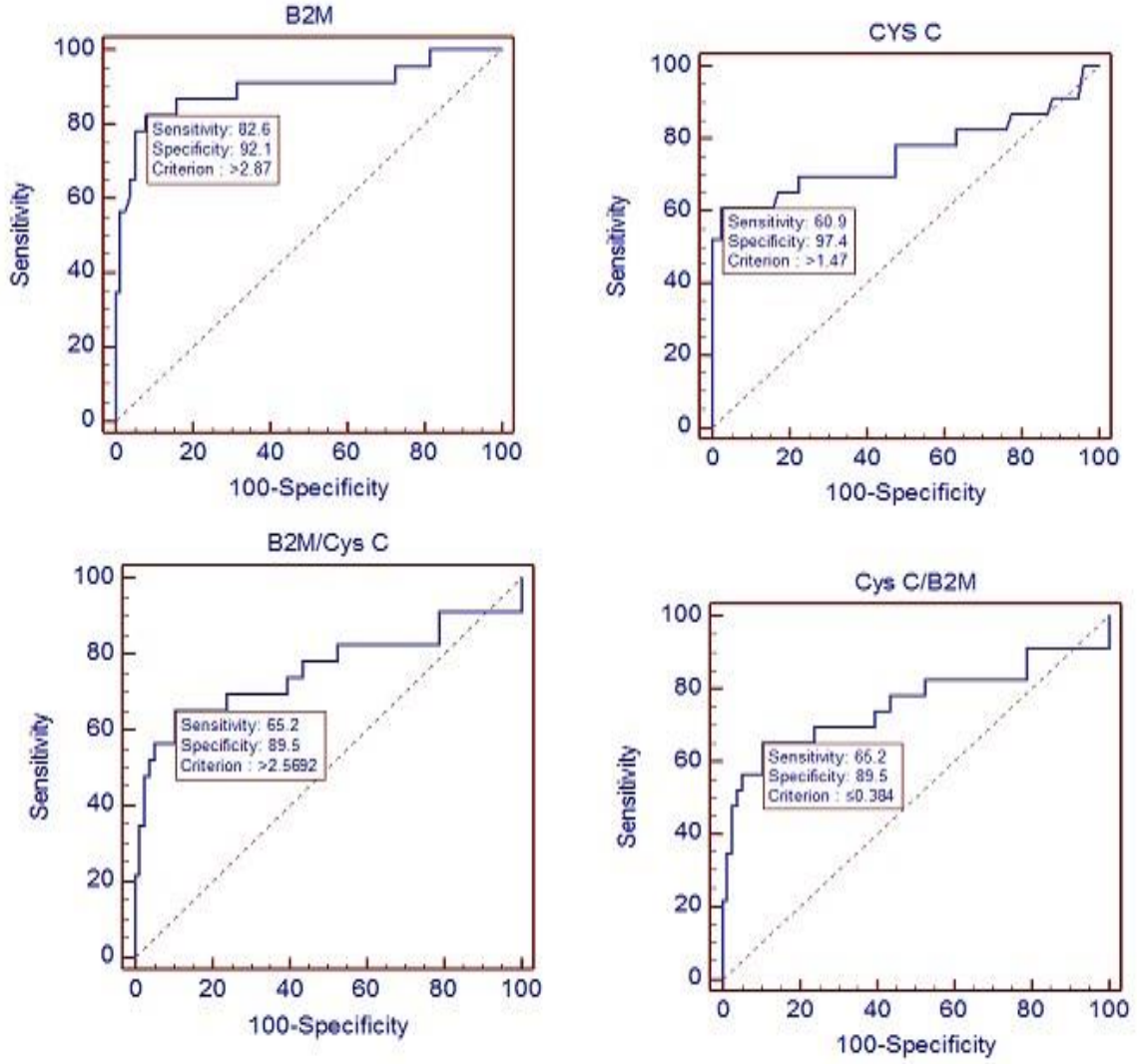

Figure 4.9 Receiver operating characteristic (ROC) curve of biomarkers in predicting CKD among participants with both DM and HTN

Diagnostic accuracy of cystatin C, B2M, B2M/cystatin C ratio and cystatin C/B2M ratio are shown in **Table 4.8.** Overall B2M was the most accurate biomarker in predicting CKD irrespective of DM, HTN or both DM+HTN condition.

Among participants with DM the best marker for predicting CKD was B2M at a cut-off point of >3.00mg/ml [AUC=1.000(0.877 to 1.000), sensitivity (100.0%), specificity (100.0%); p<0.0001] followed by cystatin C (cut-off=>1.440mg/ml, AUC=0.861(0.678 to 0.962), sensitivity (60.0%), specificity (100.0%), p=0.0002] and the combine biomarkers [AUC=0.765(0.568 to 0.903), sensitivity (80.0%), specificity (82.6%), p=0.1104].

Among participants with HTN the best marker for predicting CKD was B2M at a cut-off point of >2.10mg/ml [AUC=0.873(0.755 to 0.947), sensitivity (81.82%), specificity (84.09%); p<0.0001] followed by cystatin C (cut-off=>0.96mg/ml, AUC=0.731(0.595 to 0.842), sensitivity (72.73%), specificity (77.27%), p=0.0401] and the combine biomarkers [AUC=0.614(0.473 to 0.742), sensitivity (36.36%), specificity (93.18%), p=0.3037].

Among participants with DM+HTN the best marker for predicting CKD was B2M at a cutoff point of >2.87mg/ml [AUC=0.896(0.819 to 0.948), sensitivity (82.61%), specificity (92.11%); p<0.0001] followed by cystatin C (cut-off=>1.47mg/ml, AUC=0.758(0.661 to 0.838), sensitivity (60.87%), specificity (97.37%), p=0.0006] and the combine biomarkers [AUC=0.757(0.660 to 0.837), sensitivity (65.22%), specificity (89.47%), p=0.0005].

Table 4.8. Diagnostic accuracy of B2M, Cys C, B2M/Cys C ratio and Cys C/B2M ratio for predicting CKD among participants with DM, HTN and DM+HTN

Conditions	Markers	AUC (95% CI)	Sensitivity	Specificity	Associated criterion (Cut-off point)	P-value
DM	B2M	1.000(0.877 to 1.000)	100.0%	100.0%	>3.000	<0.0001
	Cys C	0.861(0.678 to 0.962)	60.0%	100.0%	>1.440	0.0002
	B2M/Cys C ratio	0.765(0.568 to 0.903)	80.0%	82.6%	>2.661	0.1104
	Cys C/B2M ratio	0.765(0.568 to 0.903)	80.0%	82.6%	≤0.369	0.1104
HTN						
	B2M	0.873(0.755 to 0.947)	81.82%	84.09%		<0.0001
	Cys C	0.731(0.595 to 0.842)	72.73%	77.27%	>2.1	0.0401
					>0.96	
					>2.698	
	B2M/Cys C ratio	0.614(0.473 to 0.742)	36.36%	93.18%	≤0.3649	0.3037
	Cys C/B2M ratio	0.614(0.473 to 0.742)	36.36%	93.18%		0.3037
DM+HTN	B2M	0.896(0.819 to 0.948)	82.61%	92.11%	>2.87	<0.0001
	Cys C	0.758(0.661 to 0.838)	60.87%	97.37%	>1.47	0.0006
	B2M/Cys C ratio	0.757(0.660 to 0.837)	65.22%	89.47%	>2.5692	0.0005

Cys C/B2M ratio	0.757(0.660 to 0.837)	65.22%	89.47%	≤0.384	0.0005

AUC: Area under the curve

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CHAPTER 5

DISCUSSIONS

5.1 CLINICAL CHARATERISTIC AND BIOCHEMICAL PROFILE

The main objective of the study was to evaluate the value and possible clinical applications of cystatin C and Beta 2 microglobulin as biomarkers for fluctuations in glomerular filtration rate (GFR). This was important because findings could help clinicians in early detection of chronic kidney disease (CKD), as well as avoiding misclassification of CKD patients in Ghana.

In the light of the limitations of serum creatinine (SCr) in the early detection of chronic kidney disease, there has been an ardent search for more sensitive biomarkers for CKD (Westhuyzen, 2006). This has led to the discovery of cystatin C and Beta 2 microglobulin as candidate biomarkers for early diagnosis of impaired renal function (triggered by either diabetes and or hypertension). It is an established fact that creatinine exceeds normal levels only when 50% of glomerular filtration rate (GFR) has already been lost, by which time the CKD patient may be approaching the latter stages of renal disease (Petri et al., 1988; Perrone *et al,*. 1992); thus making it a poor candidate indeed for early detection of CKD. According to Perrone et al. (1992), creatinine's relationship with muscular mass is what accounts for its inability to be sensitive to small changes in GFR; this perhaps explains why creatinine lacks sensitivity in populations with lower muscular mass, especially the older population (Lamb et al., 2003). This view agrees with that of Roos et al. (2007) who held that it is hard for SCr to detect mild to moderate renal dysfunction.

This study was conducted using an older population of 182 individuals as evidenced by the mean age of 57.59 years shown in Table 4.1; however, majority of the participants were between ages 60 and 79 years. The mean age observed to some extent does agree with that

reported (57.68±10.39) by Amartey et al (2015). Per the aforementioned, these data would have implications on the ability of creatinine to effectively diagnose participants of CKD; hence, the need for biomarkers such as Cystatin C and B2M. Since the difference in the mean ages of the genders was found to be statistically insignificant (Table 4.1), it can be safely deduced that ages of both males and females may not be a confounding variable in this study because of the occurrence of homogeneity, age-wise with respect to gender. As such findings made may be applicable to individuals irrespective of age of individuals of a particular gender.

Diabetes and hypertension are two of the most important factors accounting for cardiovascular disease development (White, 2007), which in itself is a major precursor for CKD. Study results indicated that majority (54.4%) of the participants had both diabetes and hypertension (DM+HTN), followed by hypertension (HTN) only (30.2%) and diabetes only (DM) (15.4%) (**Table 4.1**). The frequency of hypertension observed for this study is similar to the 35.4% observed reported by Fox et al (2010). This data seems to suggest a strong connection between diabetes and hypertension, in that there were more people who had both conditions than they were that had only a single condition. This assertion is supported by

(White, 2007), who opined that "diabetes and hypertension…rarely exist in isolation" (p. 365). He further went on to explain that the two conditions are inseparably linked, indicating that majority of persons with diabetes also suffer from hypertension and that a considerable number of those suffering from the latter later develop the former as well. This is further supported by findings from Mahmood et al (2010), who observed that of the 80 participants used for the study, the majority (36.67 %; n = 11) had both diabetes and hypertension. This view is also supported by findings that people with cardiovascular disease conferred by hypertension are more likely to develop diabetes, which is an important risk factor for CKD.

Glomerular filtration rate is the best estimate of the number of functioning nephrons and functional renal mass (Mahmood et al., 2010; Zhang et al., 2014). Accurate measurement of glomerular filtration rate (GFR) is costly and laborious, as such several filtered substances, such as urea, uric acid, creatinine, cystatin c and B2M could be used in the estimation of GFR (Humes, 1997). End-stage renal failure in all forms of progressive kidney disease is associated with a lowering of GFR levels; hence, knowledge of GFR is critical in the prevention and management CKD (Street., 2008). Only mean levels of total protein and uric acid in both gender (Table 4.1) respectively exceeded normal levels of total protein (6086g/L) and uric acid (males: 214-486 mmol/L; females: 137-363). However, observed mean levels of Cr, urea, albumin, Cys C and B2M all fell within the reference range, suggesting that these filtered substances may not give equal response to changes in GFR. Further analysis revealed that there was the occurrence of significantly elevated mean levels of Cr, uric acid, cystatin C and B2M in males compared to females ($p<0.05$) (Table 4.1). This may probably be due to susceptibility of the male gender to CKD, as the male gender is associated with a more rapid progression of renal disease (Mahmood et al., 2010).

Both diabetes and hypertension trigger different biochemical characteristics in participants, despite the strong linkage between diabetes and hypertension as seen in Table 4.2, which showed that there was a statistically significant difference between the means age, total protein, uric acid, eGFR (both criteria), and EPI-Cr-Cys C levels among participants with DM, HTN and both DM + HTN ($p<0.0001$). The fact that participants with DM + HTN and only HTN were significantly older than those with only DM ($p<0.0001$) seem to suggest that:

(1) development of diabetes may precede hypertension; (2) Individuals in their late fifties seem to be more susceptible to HTN only as well as both conditions (DM + HTN). These statements are buttressed by studies which indicate that prevalence of hypertension was 54% higher in

diabetic persons (Pell and D'Alonzo, 1967). Furthermore, after reviewing several literature on diabetes and hypertension, Fuller (1985) concluded that "the balance of evidence points to increased frequency of hypertension in diabetic populations. This increase is mainly in the older age groups." (p. II-5).

Additionally, the fact that eGFR was significantly ($p<0.05$) elevated among participants presenting with only DM compared to both DM+HTN and only HTN is an indication that renal function is probably more impaired in participants with both DM+HTN and only HTN compared to DM only (Table 4.2), considering the fact that the lower the GFR, the greater the severity of glomerular damage and by extension the CKD. Data in Figure 4.3 provide further support in that higher proportion of the participants with both DM+HTN had CKD, when compared to those with DM only and HTN only. The collected data in Table 4.2 seem to further suggest that hypertension may be a major factor in advancing CKD in diabetics. This view is corroborated by the finding that serum uric acid levels was significantly elevated among participant with both DM+HTN compared to those with only HTN ($p<0.0001$).

5.2 CKD STAGING OF STUDY PARTICIPANTS

Glomerular filtration rates (GFR) was estimated using both the Modification of Diet in Renal Disease (MDRD) and the CKD-EPI formula. This methodology was also used by Delanaye et al. (2010) in a study to determine if there were any observable differences in prevalence of stage 3 CKD as a result of using either MDRD or the CKD-EPI formulae. Delanaye et al. (2010) observed that prevalence of stage 3 CKD in a Belgian population aged between 45 and 75 years using the MDRD or the CKD-EPI equations was 11.04 and 7.98%, respectively. On the contrary, prevalence of stage 3 CKD as observed in this study was higher (Figure 4.1) than that reported by Delanaye et al. (2010).

Furthermore, from the results, the percentage of participants with CKD stage 1, 2 and 3a were higher using the MDRD criteria in relation to CKD-EPI; whilst, a higher number of participants had CKD stage 3b and 4 using CKD-EPI equation compared to using the MDRD equation (Figure 4.1). This presupposes that the MDRD criteria may be more sensitive in detecting early stages of CKD (1-3a) than latter stages (3b-5), whilst vice versa for the CKDEPI criteria. Results in Table 4.3 and Figure 4.3 provide some support to this assertion. From Figure 4.3, it can be seen that using the MDRD equation a higher proportion of participants with stage 3 CKD was detected compared to CKD-EPI. Delanaye et al. (2010) also noted that the prevalence of stage 3 CKD is significantly higher with the MDRD study equation ($p < 0.0012$), thus lending credence to earlier assertion. The only issue with Delanaye's (2010) findings however is that we don't know for sure whether the prevalence of stage 3 CKD refers more to 3a or to 3b. Working on prevalence and determinants of CKD, Rothenbacher et al. (2012) found that prevalence of CKD stage 3–5 was 34.3% by MDRD, 33.0% by CKDEPI, and 14.6% by the CysC based estimating equations.

5.3 EFFECT OF AGE RACE AND GENDER ON CYSTATIN C

Gender has always been an important issue in CKD, partly because of their influence on levels of cystatin C and creatinine (Wasen et al., 2003; Keller et al., 2007). For example, results in Table 4.1 indicate that levels of both Cr and Cys C were significantly lower in females compared to males. Further analysis show that level of Cys C in females was 16.79 % lower than that in males, a figure higher than the 9 % reported by Stevens et al. (2008b).

But then, Stevens et al.'s (2008b) figure was reported for 40 year olds whilst that of this study was reported for females in their fifties. Age then seems to play a key role in biomarker levels. This seem suggest that gender and age has effect on both cystatin c and creatinine. The effect of gender, age and race on creatinine levels are well documented. Cystatin C until recently was

widely reported not to be affected by age, race and gender one which was reported by Groesbeck et al. (2008). However new data available seem to suggest an association between gender age race on Cystatin C (Johnston et al; 2004) (Knight et al; 2004). Findings from this also seem to point to gender having an effect on Cystatin C. Data collected indicate that there was a significant association between GFR and age (Table 4.3), as well as a significant negative correlation between CKD and age (Table 4.6). As important as gender is, there was no significant association between GFR and gender irrespective of the GFR equation used ($p > 0.05$) (Figure 4.2), perhaps this was because there was no statistically significant difference in the mean ages of both males and females.

5.4 CORRELATIONAL STUDIES BETWEEN BIOCHEMICAL PROFILE AND BIOMARKERS

The fact that mean levels of Cys C and B2M were significantly elevated among participants with CKD when compared to those without CKD ($p < 0.0001$) as shown in Figure 4.4 is an indication that elevated levels of Cys C and B2M are diagnostic of damage to the kidney. The consistent negative correlation between Cystatin C, Beta 2 microglobulin and eGFR indicates the importance of these markers in diagnosing renal damage at any level These results agree somewhat with findings in other studies such as Rocha et al. (2014). Additionally, general study participants with were found to be significantly older than those without CKD, suggesting that CKD are more associated with older persons. Further analysis also revealed that those with CKD had significantly elevated mean levels of Cr, uric acid, urea, total protein and albumin, a confirmation of the usage of these filtrates in the determination of GFR.

Correlational studies show that there exists between CKD and urea, uric acid, cystatin C and

B2M among participants with both DM+HTN a significant negative correlation as seen in Table 4.6, suggesting that prognosis of CKD in persons with both DM and HTN can best be done by monitoring the flow of these GFR filtrates: urea, Cys C and B2M. Uric acid was left out because it failed to hold when the partial Pearson correlation was adjusted for age.

It appears that even without CKD, diabetics and hypertensives may still be at risk of later developing CKD. This assertion is grounded in the fact that GFR significantly correlated negatively with age (r= -0.705; p=0.0002), and cystatin C (r= -0.587; p=0.003) among diabetics *without CKD*; whilst, among hypertensive (HTN) participants *without CKD*, GFR significantly correlated negatively with age (r= -0.337; p=0.025), total protein (r= -0.361; p=0.016), cystatin C (r= -0.339; p=0.025) and Beta 2 Microglobulin (r= -0.429; p=0.004) as seen in Table 4.6. Moreover, it appears that Cys C is able to significantly correlate with GFR, where B2M fails. A case in point is the significant correlation of Cys C with GFR among diabetics *without CKD*. However, further analysis using partial Pearson correlation coefficient indicate this not to be the case, in that after adjusting for age, the correlations: cystatin C vs No CKD in DM, and cystatin C vs No CKD in HTN failed to hold; thus making Beta 2 Microglobulin more pervasive and so more reliable as a biomarker for CKD

prognosis.

5.5 DIAGNOSTIC ACCURACY OF BIOMARKERS

Receiver Operating Characteristics (ROC) are commonly used to summarise the classification accuracy of diagnostic test. Diagnostic accuracies of cystatin C, B2M,

B2M/cystatin C ratio and cystatin C/B2M ratio were assessed by ROC curves as shown in **Table 4.8.** In clinical practice serum creatinine and creatinine clearance are widely used as indirect markers makers for GFR. GFR is commonly estimated using serum creatinine using

the MDRD study equation. Serum levels of endogenous filtration markers and eGFR derived from markers are accurate index of measured GFR in steady state conditions. It was observed by Donadio et al. (2001) that cystatin c and beta-2- microglobulin had diagnostic accuracies very similar to creatinine as markers of GFR In this study, overall B2M was the most accurate biomarker in predicting CKD irrespective of DM, HTN or both DM+HTN condition. However, diagnostic accuracy of B2M occurs at varying cut-off points. Among participants with DM the best marker for predicting CKD was B2M at a cut-off point of >3.00mg/ml [AUC=1.000(0.877 to 1.000), sensitivity (100.0%), specificity (100.0%); p<0.0001]; with regards to participants with HTN the best marker for predicting CKD was also B2M at a cut-off point of >2.10mg/ml [AUC=0.873(0.755 to 0.947), sensitivity (81.82%), specificity (84.09%); p<0.0001]; pertaining to participants with DM+HTN again the best marker for predicting CKD was B2M at a cut-off point of >2.87mg/ml [AUC=0.896(0.819 to 0.948), sensitivity (82.61%), specificity (92.11%); p<0.0001]. In a study done by Aksun et al. (2004) in type 2 diabetics, diagnostic accuracies of Cystatin C and

Beta 2 microglobulin were assessed by ROC curves. The area under the curve for serum Cystatin C was 0.830 and for Beta 2 microglobulin was 0.810. Tubular involvement of B2M may precede glomerular involvement because several of the tubular proteins and enzymes are detectable even before the appearance and rise in serum creatinine. B2M has been advocated as a better predictor of GFR but its serum concentration can increase as a result of acute phase reactants. This may account for the higher diagnostic sensitivity of Beta- 2 microglobulin compared to Cystatin C in this study

The areas under the curve for cystatin were all significant the AUC recorded especially for diabetics and patients with both conditions is low but significant compared to a study conducted by Oddoze et al, (2002). Several other investigations have found out that cystatin c has a high

diagnostic accuracy. Discrepancies in outcomes may be as a result of environmental and ethnic differences between populations or differences in sample collection and storage. Another reason of the lower AUC of cystatin c in this study compared to other studies might be that effect of high level of triglycerides on cystatin c. Dyslipidemia is a complication of CKD associated with high triglyceride levels (Sowers, 2007). A few studies have shown like as reported by Witzel et al. (2014) that elevated triglyceride levels may decrease serum cystatin c levels.

The combined biomarkers in this study did not register any significant AUC index. It is not uncommon in medical practice that multiple diagnostic test or markers are combined to find their diagnostic accuracies. The insignificance of the AUC index in this study for diabetics and hypertensives only contrast studies that have been done that shows that the AUC index for combined markers better than each single markers. A study done by Stein et al (2009) combining urinary albumin/creatinine ratio and eGFR substantially improved diagnostic accuracy with an AUC of 0.936. So in this study combined biomarkers are of less diagnostic value to be used for prognosis of CKD in persons with Diabetes only or persons with hypertension only.

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CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

The diagnostic accuracy of biomarkers may vary depending on the conditions in question, coupled with the attendant biochemical profiles of individuals in question. The study shows that serum Beta 2 Microglobulin and serum cystatin c have acceptable and significant sensitivities and specificities in detecting renal impairment

The study also shows that there is a strong association between diabetics and hypertensives. There is an increasing an increasing number of people with both Diabetes and hypertension

Among diabetics with CKD, hypertensives with CKD and participants displaying both conditions (DM+HTN) and have CKD, there was no significant correlation between, Cystatin C vs. Creatinine and Beta 2 Microglobulin vs. Creatinine. Among participants with both conditions and have CKD, there was negative significant correlation between Cystatin C vs eGFR, and B2M vs. eGFR. Additionally, in hypertensives with CKD, there was a significant negative correlation between B2M and eGFR. Cys C showed a significant correlation with eGFR among persons without CKD, regardless of condition. Nonetheless, B2M showed a significant negative correlation with eGFR among hypertensives only, as well as those with both diabetes and hypertension.

Findings also show that in participants with diabetes only and also those with hypertension only, the combined biomarkers did not register any significant AUC index; and so combined biomarkers are of less diagnostic value to be used for prognosis of CKD in persons with

Diabetes only or persons with hypertension only.

6.2 RECOMMENDATION

It is recommended that any related study should utilise measured GFR using exogenous substances to appropriately compare the sensitivities of the various markers. It is also recommended that subsequent studies find out the correlation between cystatin c and triglycerides.

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