PREDICTIVE EQUATIONS, OXIDATIVE AND METABOLIC RISK FACTORS AMONG GHANAIAN PATIENTS PRESENTING WITH 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. This work has not been submitted for any other degree.

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ABSTRACT

Current recommendations emphasize the need to assess kidney function using creatinine-based predictive equations to optimize the care of patients presenting with chronic kidney disease. The most widely used equations are the Chronic Kidney Disease-Epidemiology Collaboration (CKD-EPI), Cockcroft-Gault (CG) and the simplified Modification of Diet in Renal Disease (MDRD) formulae. However, none of the predictive equations have been validated for the assessment of chronic kidney disease (CKD) cases in Ghana. The metabolic syndrome (MetS) is a common risk factor for cardiovascular and chronic kidney disease (CKD) in Western populations. The relationship between metabolic syndrome and risk of CKD in underdeveloped countries where genetic and environmental backgrounds differ from those in Western countries is not known. Anaemia, a complication of CKD is a potential nontraditional risk factor for cardiovascular disease (CVD). Dyslipidaemia and lipid peroxidation are both known risk factors for cardiovascular disease. This study assessed the lipid profile and oxidative stress/lipid peroxidation in patients presenting with Chronic Kidney Disease (CKD) using the oxidative stress marker; Malondialdehyde (MDA) and antioxidants; Vitamins A and C, Catalase and Uric Acid. Parathyroid hormone (PTH) has been identified as the main regulator of some electrolytes homeostasis, and thus this study set out to evaluate the relationship between PTH and these electrolytes as well as their ratios. The overall aim of this study was to evaluate the use of renal function equations in the assessment of renal function in CKD and to identify specific oxidative and metabolic risk factors in CKD. This is, therefore, the first study to specifically evaluate the predictive performance and accuracy of the seven renal function equations in patients presenting with CKD in our community. Furthermore, this study evaluated whether anaemia poses a cardiovascular risk and whether the risk is modified by the presence of CKD. In addition the present study sought to examine the association between the metabolic syndrome and risk of CKD among Ghanaian patients presenting with CKD. This study also assessed the lipid profile and oxidative stress/lipid peroxidation in patients presenting with CKD using the oxidative stress marker; Malondialdehyde (MDA) and antioxidants; Vitamins A and C, Catalase and Uric Acid. Finally, the relationship between PTH and electrolytes as well as their ratios was evaluated. Anaemia was defined as haemoglobin concentration ≤ 11.0 for both males and females whereas CKD was defined as an estimated GFR of ≥ 60 ml/min per 1.73 m^2.

The study population included 146 individuals with various diagnosed chronic kidney diseases. Another 80 healthy subjects without any chronic kidney pathology but of similar age and sex distribution were used as controls.

The results of these predictive equations for 146 patients using stage of CKD were compared with the recommended methods (4v-MDRD and CKD-EPI). The MetS was defined as the presence of three or more of the following risk factors according to the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) criteria: elevated blood pressure, low high density lipoprotein cholesterol (HDL-C), high triglycerides, elevated plasma glucose and abdominal obesity. Anaemia was defined as haemoglobin concentration ≤ 11.0 for both males and females whereas CKD was defined as an estimated GFR of ≥ 60 ml/min per 1.73 m^2.

The most accurate results were obtained with the reference equations (4v-MDRD and CKD-EPI) with CKD-EPI having a slight edge over 4v-MDRD equation. The sensitivity and specificity of the 4v-MDRD equation to detect glomerular filtration rate (GFR) values < 60 ml/min/1.73 m^2 were 50.0% and 60.0% respectively; that of CKD-EPI was 66.6% and 70.0% respectively.

The prevalence of MetS among CKD subjects in this study was 30.1%. The CKD groups had significantly higher waist circumference (WC), were more hypertensive [based on systolic blood pressure (SBP) and diastolic blood pressure (DBP)], had more diabetics based on fasting blood glucose (FBG) and were more hypercholesterolaemic and hypertriglyceridaemic (i.e. TC and TG) as compared to the control. The CKD group are also about 9 times at risk of developing MetS as compared to the control group (OR = 8.8; 95% CI = 3.8-20.5). The female subjects with CKD are 2 times at risk of developing metabolic syndrome as compared to the male counterparts (OR = 1.9; 95% CI = 0.9-4.0). The CKD patients were about 9 fold at risk of developing hypertension (OR = 8.9; 95% CI = 3.1- 25.1) and diabetes (OR = 9.3; 95% CI = 4.7-18.2), about 2 times at risk of developing hypertriglyceridaemia (OR = 2.3; 95% CI = 1.3-4.2) and several folds at risk of developing proteinuria (OR = 409; 95% CI = 24.7-6759). There was a significant graded relationship between the number of MetS components present and risk of CKD. 58.9% of the subjects had CKD with an estimated GFR (eGFR) of < 60 ml/min/1.73 m^2, estimated with the Modification of Diet in Renal Disease (MDRD) equation and were more likely to be anaemic and nondiabetic, with higher mean values for serum creatinine (CRT) lower values for haemoglobin (HGB), haematocrit (HCT), and red blood cells (RBC). CKD subjects with anaemia had a higher prevalence of several cardiovascular (CVD) risk factors; age, male sex, diabetes and hypertension and lower haematological parameters and estimated GFR. However they had higher total cholesterol (TC) and higher triglyceride (TG) level. With the exception of HDL-C, which showed no significant difference when CKD patients were compared with controls, total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), and triglycerides (TG) increased significantly in the CKD patients. Serum MDA increased significantly in the CKD patients as compared to the controls and increased with the severity of the condition. Vitamin A, Catalase and Uric Acid increased significantly in the CKD subjects as compared to controls, whilst vitamin C decreased significantly among the CKD subjects. For every mmol/l increase in the serum concentration of PO_4^{2-} ($r^2 = 0.78$, $p < 0.0001$), K^+ ($r^2 = 0.28$, $p < 0.0001$)

and Mg^{2+} ($r^2 = 0.004$, $p = 0.0211$) there was a corresponding increase in serum concentration of PTH with beta values of 0.005, 0.0007 and 0.001, respectively. However, there was no linear relationship between Na^{+} and PTH ($r^2 = 0.001$, $p = 0.6687$). The serum concentration of PTH decreased, for every mmol/l increase in the serum concentrations of Ca^{2+} ($r=0.33$, $p < 0.0001$).

These results suggest that measurement of GFR with predictive equations might be a prudent strategy for the assessment of renal function among the CKD population and that the metabolic syndrome might be an important factor in the cause and progression of chronic kidney disease among Ghanaian patients presenting with CKD. Furthermore, in persons with CKD, anaemia poses a further cardiovascular risk as it increases some of the traditional cardiovascular risk factors. Dyslipidaemia and increased oxidative stress with abnormal antioxidant levels are common in CKD patients. Therapeutic regimens aimed at strengthening the antioxidant defenses as well as normalizing lipid concentrations would be useful in protecting CKD patients against oxidative stress and any related complications. Excess PTH is linked with derangements in the metabolism of electrolytes like calcium, magnesium, phosphorus and potassium in CKD and contributes to a plethora of complications.

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ABBREVIATIONS

Adj Ca	Adjusted Calcium
ADMA	Asymmetric Dimethyl Arginine
ANOVA	Analysis of Variance
BJ	Bjornsson
BMI	Body Mass Index
BSA	Basal Surface Area
BUN	Blood Urea Nitrogen
Ca/K	Calcium-Potassium Ratio
Ca/Mg	Calcium- Magnesium Ratio
Ca/Na	Calcium-Sodium Ratio
CAT	Catalase
^{51}Cr-EDTA	Chromium-51-Ethylene Diamine Tetraacetic Acid
Ccr	Creatinine Clearance
CG	Cockcroft- Gault
CHD	Coronary Heart Disease
CHRPE	Committee on Human Research Publication Ethics
CI	Confidence Interval
CKD	Chronic Kidney Disease
CKD-EPI	Chronic Kidney Disease-Epidemiology Collaboration
CRT	Creatinine
CVD	Cardiovascular Disease
DBP	Diastolic Blood Pressure
DNA	Deoxyribonucleic Acid
DTC	Dinitrophenylhydrazine Thiourea Copper Sulphate
ECF	Extracellular Fluid
eGFR	Estimated Glomerular Filtration Rate

ESKD	End Stage Kidney Disease
ESRD	End Stage Renal Disease
FBG	Fasting Blood Glucose
GFR	Glomerular Filtration Rate
GT	Gates
HCT	Haematocrit
HDL-C	High Density Lipoprotein Cholesterol
HGB	Haemoglobin
HPLC	High Performance Liquid Chromatography
HT	Height
IDF	International Diabetic Federation
JL 1	Jelliffe 1
JL 2	Jelliffe 2
K/DOQI	Kidney Disease Outcomes Quality Initiative
K/Mg	Potassium-Magnesium Ratio
K_2 EDTA	Dipotassium Ethylene Diamine Tetraacetic Acid
KATH	Komfo Anokye Teaching Hospital
LDL-C	Low Density Lipoprotein Cholesterol
LV	Left Ventricular
LPD	Low Protein Diet
MDA	Malondialdehyde
4v-MDRD	4 -Variable Modification of Diet in Renal Disease
MS	Metabolic Syndrome
Na/K	Sodium-Potassium Ratio
Na/Mg	Sodium-Magnesium Ratio
NCEP ATP III	National Cholesterol Education Program, Adult Panel III

NKF-KDOQI	National Kidney Foundation- Kidney Disease Outcomes Quality Initiative
NO	Nitric Oxide
NF-B	Nuclear Transcription Factor -B
OR	Odds Ratio
PRT	Proteinuria
PTH	Parathyroid Hormone
RAS	Renin Angiotensin System
RBC	Red Blood Cell
Rho-ROCK	Rho-associated coiled-coil kinase
RNA	Ribonucleic Acid
ROC	Receiver Operator Characteristic
SBP	Systolic Blood Pressure
Scr	Serum Creatinine
SEM	Standard Error of the Mean
SGA	Subjective Global Assessment
SHPT	Secondary Hyperparathyroidism
SOLVD	Studies of Left Ventricular Dysfunction
^{99m}Tc-DPTA	Technetium Labeled Diethylene-Triamine-Pentacetate
TBA	Thiobarbituric acid
TC	Total Cholesterol
TG	Triglyceride
USRDS	United States Renal Data System
W.H.O.	World Health Organization
WC	Waist Circumference
WT	Weight

Chapter 1

INTRODUCTION

1.1 GENERAL INTRODUCTION

Chronic kidney disease (CKD) is a worldwide health problem, affecting millions of people (Di Angelantonio *et al.*, 2007). The magnitude of the problem is poorly described by the number of people that will initiate renal replacement therapy (haemodialysis, peritoneal dialysis and renal transplantation), as the incidence of 1-3 per 10,000 per year in the general population may seem small (Lysaght, 2002; Hallan *et al.*, 2006; Dor *et al.*, 2007; Hsu *et al.*, 2007). However, chronic dialysis treatment and transplantation have an enormous impact on the life of individual patients and their families, and renal replacement therapy is very costly (Lysaght, 2002; Dor *et al.*, 2007). The annual worldwide costs are estimated at 70 to 75 billion US dollars to maintain the renal replacement therapy of the roughly 1.1 million worldwide dialysis patients in 2001. In Ghana, renal replacement therapy (mainly haemodialysis and peritoneal dialysis) is available only in two of the teaching hospitals, and the estimated cost of dialysis is GHC 57,600 (approximately $44,300) per patient per annum. This amount is rather high for a country with a per capita income of $1500 and a GDP of 6.3%. The first renal transplant in this country was performed at the end of 2008 by a combined team of Ghanaian and British surgeons. Moreover, the number of patients requiring renal replacement therapy is increasing globally, by up to 7% annually according to some reports (Gansevoort *et al.*, 2004; Jones *et al.*, 2005; Muntner *et al.*, 2005).

CKD represents a progressive irreversible decline in the glomerular filtration rate (GFR). A common phenomenon in renal failure is progressive renal function loss irrespective of the underlying cause of the kidney disease. Most chronic nephropathies lack a specific treatment and progress relentlessly to end stage kidney disease (ESKD), which prevalence is increasing worldwide (Locatelli *et al.*, 2003b). The underlying cause of CKD has shifted from classic causes such as

glomerulonephritis and interstitial nephritis, to atherosclerosis and diabetic nephropathy. In contemporary times the latter two accounts for 40%-50% of cases as primary diagnosis in many countries (Friedman *et al.*, 2006; Friedman and Friedman, 2007).

The most common screening test for CKD is the measurement of serum creatinine. However, it as an insensitive measure, since as much as 50% of the nephron mass may be lost before creatinine concentration increases and levels are influenced by several factors such as sex, age, body mass, and diet (Parmar, 2002).

The National Kidney Foundation Dialysis Outcome Quality Initiative (NKF/DOQI) (National Kidney Foundation, 2002) and European Best Practice Guidelines (European Best Practice Guidelines for Haemodialysis, 2002) recommend the use of prediction equations to estimate the GFR from serum creatinine. In adults, the most commonly used formulae are those derived from the Modification of Diet in Renal Disease (MDRD) study population (Levey *et al.*, 1999a) and that by Cockcroft and Gault (Cockcroft and Gault, 1976). The MDRD equations were derived from patients with varying degrees of renal impairment employing a stepwise regression technique, with GFR measured from the renal clearance of [^{125}I] iothalamate (Levey *et al.*, 1999a). In its original form, the MDRD formula used six variables (serum creatinine, albumin and urea nitrogen, gender, age and ethnicity), although two simpler equations requiring either five variables (excluding serum albumin) or four variables (excluding serum albumin and urea nitrogen) were proposed to offer a similar performance (Levey *et al.*, 1999b). The Cockcroft and Gault formula, in marked contrast, was constructed from hospitalized patients to predict creatinine clearance from the serum creatinine in the absence of urine collection (Cockcroft and Gault, 1976). The NKF has suggested the following definition of CKD: established kidney damage with structural or functional abnormalities or a glomerular filtration rate <60 ml/min/1.73 m^2 for three months or more (National Kidney Foundation, 2002). The classification of stages of CKD is based on the level of kidney function measured by GFR, where

stage 1 represents kidney damage with normal or elevated GFR and stage 5 represents a GFR of less than 15 ml/min or who require treatment with dialysis. In recent times a new equation the Chronic Kidney Disease-Epidemiological Collaboration (CKD-EPI) has been introduced (Levey *et al.*, 2009). The CKD-EPI equation has lower bias, especially at an estimated GFR greater than 60 mL/min per 1.73 m^2; however, precision remains limited. The enhanced accuracy of the CKD-EPI equation overcomes some of the limitations of the MDRD study equation and has important implications for public health and clinical practice.

Table 1.1 Stages of CKD according to National Kidney Foundation.

Stage	Description	GFR (mL/min/1.73 m2)
1	Kidney damage with normal or increased GFR	≥90
2	Kidney damage with mildly decreased GFR	60-89
3	Moderate kidney disease	30-59
4	Severe kidney disease	15-29
5	Kidney failure	<15 or dialysis

1.2 FUNCTIONS OF THE KIDNEYS

The kidneys play an important role in the maintenance of normal body function.

The basic function of the kidneys has to do with the formation of urine through complex filtration, reabsorption and secretion mechanisms. In addition, the kidneys also excrete urea and uric acid which are the end products of protein and nucleic acid metabolism.

The kidneys regulate fluid, electrolyte and acid base balance of the body and create a steady environment for the metabolic processes of tissues and cells. This function is essential for life and it is realized by balancing solute and water transport, excreting metabolic waste products, conserving nutrients, and regulating acid-base balance in the body.

Additionally, the kidney also produces three (3) important hormones; erythropoietin which stimulates the production of red blood cells, renin which regulates blood pressure and calcitriol (the active form of vitamin D) which helps in regulation of plasma calcium levels.

1.3 DEFINITION OF KIDNEY DISEASE

Kidney disease results in the loss or reduction of functioning nephrons.

Chronic kidney disease (CKD) a new terminology that has replaced chronic renal failure (CRF) was defined in 2002 by the National Kidney Foundation Kidney Disease Quality Outcome Initiative (NKF/KDOQI) as structural damage or GFR<60 ml/min/1.73 m^2 for more than three months. Kidney damage is defined by the NKF/KDOQI as pathological abnormalities or markers of kidney damage, including abnormalities in urine or blood tests or abnormal imaging tests (NKF/KDOQI™, 2002).

This new vocabulary provides a platform for healthcare professionals tasked with caring for CKD patients worldwide to speak a common language. In addition, it gives a simple definition of CKD and a staging system which distinguishes groups of patients (Levin, 2003).

1.3.1 Prevalence of CKD

The prevalence of CKD in the western societies (United States, Britain and parts of Asia) ranges between 5-15% in the adult population (Chen *et al.*, 2011). In 2009 Afolabi and his colleagues put the prevalence among Nigerians in a family practice population at 10.7% (Afolabi *et al.*, 2009). The prevalence of CKD in Ghana has varied over the years; from 1.6% per million people by Bamgboye (2006) to 4% among hypertensives in the Greater Accra region as documented in the study by Ado *et al.*, (2009). Recently, a prevalence of 46.9% has been recorded among hypertensives in Ghana (Osafo *et al.*, 2011).

1.4 RISK FACTORS OF CKD

Clinical and epidemiological reports have provided a relationship between numerous factors and the initiation and progression of CKD. These have been grouped into two well defined classes: those that cause the CKD (risk factors) and those that are associated with CKD in the absence of established causal relations (risk markers).

1.4.1 Aetiology and risk factors

Progressive glomerulopathies are among renal diseases which cause a rapid permanent loss of kidney function. Most kidney diseases progress slowly over ten to fifteen years, initially without symptoms. This makes it very difficult to identify the aetiology. Indications are that environmental and lifestyle factors affect kidney function even though genetic factors also show some relevance (Bowden, 2003). Generally, kidney function reduces with age even among healthy subjects; this reduction or decline however is not similar but exhibits considerable individual variation (Lindeman *et al.*, 1985). Furthermore, there is a significant variation in the incidence of kidney damage among persons at risk for CKD (hypertension, diabetes mellitus). Finally, the rate at which kidney function is lost shows a high level of inter individual variation even among persons with the same underlying cause of kidney injury (McClellan and Flanders, 2003). Established or suspected

risk factors associated with the occurrence or progression of CKD are as shown in Table 1.2:

Table 1.2: Established or suspected factors associated with the occurrence or the progression of chronic renal failure.

Specific kidney diseases	Race and ethnicity
Hypertension	Hereditary factors
Diabetes	Low birth weight
Hyperinsulinaemia	Short stature
Chronic anaemia	Obesity
Proteinuria	Cigarette smoking
Oxidative stress	Illicit drug use
Older age	Analgesics
Male gender	High intake of proteins
Low socio-economic status	Lead, cadmium and other heavy metals
Dyslipidaemia	Organic solvent

Source: (Mitch, 2007)

1.4.2 Progression of Renal Disease

Virtually, all kidney diseases progress to terminal renal failure relatively independent of the initial disease. Diabetic nephropathy, chronic glomerular diseases and hypertensive nephrosclerosis are among the most widespresd causes of CKD (Remuzzi *et al.*, 1997). A primary disease eventually leads to secondary glomerular injury and nephron loss that is clinically characterized by proteinuria and hypertension, which leads to inflammation or scarring which causes kidney failure and ultimately a gradual elevation in the plasma creatinine concentration and a progressive decline in GFR (Jacobson, 1991). Apparently, the excessive protein filtration, caused by the glomerular hypertension, might per se have toxic effects on the kidneys and increase the rate of progression (Remuzzi and Bertani, 1998; Tryggvason and Pettersson, 2003). Studies in rats have suggested that

hyperfiltration and glomerular hypertension may play important roles (Brenner *et al.*, 1982). Hyperfiltration is observed in diabetes and obesity, but also in any condition associated with a reduced number of nephrons (Brenner *et al.*, 1996). To compensate for this nephron loss, the glomerular plasma flow rate and glomerular hydrostatic pressure increase in the surviving nephrons, thus raising the single nephron glomerular filtration rate. Initially, these changes are adaptive because they maintain the overall GFR. However, the glomerular hypertension has negative long term effects and causes progressive renal sclerosis in a self-perpetuating vicious cycle, whereby nephron loss due to sclerosis further increases flow and pressure in the remaining glomeruli leading to a gradual progress of CKD (Brenner *et al.*, 1996). The central mediator of this observed glomerular haemodynamic changes seems to be angiotensin II, but it also controls other factors that might be of importance in the progression of kidney disease, such as the production of reactive oxygen species, the regulation of cytokines and profibrotic growth factors, among others. Inappropriate activation of other systems, such as the sympathetic system, the endothelin system and of aldosterone, has also been implicated in the progression of CKD (Gross and Amann, 2004).

1.4.2.1 Hypertension

There is compelling evidence from the epidemiological studies that hypertension causes a decline in renal function (Ishida *et al.*, 2001; Young *et al.*, 2002; Fox *et al.*, 2004) and increases risks of ESKD (Perry *et al.*, 1995; Klag *et al.*, 1996). However, some investigators have questioned whether non-malignant hypertension (in contrast to malignant hypertension) is an important initiator of kidney disease (Hsu, 2002; Kincaid-Smith, 2004). Although evidence that hypertension accelerates the progression of already existing renal failure is overwhelming, there is lack of conclusive data from clinical trials that aggressive treatment of hypertension reduces risk of kidney disease onset.

1.4.2.2 Diabetes

Diabetes contributes substantially to the burden of ESKD (Brancati *et al.*, 1997; Ritz *et al.*, 1999) thus the rapidly rising trend in type II diabetes prevalence throughout the world is of major concern (Zimmet, 2003). There are indications that genetic susceptibility to nephropathy development may be in operation in both type I and type II diabetes, although gene hunting studies have been unable to identify any particular mutations which could explain why diabetic nephropathy is mostly associated with diabetic patients (Bergrem and Leivestad, 2001). Changing environmental or behavioural factors appear to be of importance for the development of diabetic nephropathy beside the genetic factors. Among Pima Indians where both type II diabetes and diabetic nephropathy are highly prevalent, the incidence rate of proteinuria among type 2 diabetics has increased nearly two fold during the last four decades, notwithstanding improvements in plasma glucose levels and blood pressure (Nelson *et al.*, 1998).

1.4.2.3 Tobacco

Tobacco belongs to the Nicotiana species. It has been used by indigenous Americans for medicinal and ceremonial purposes for many years. Early in the 20th century the habit of using tobacco as a stimulant became widespread (Routh *et al.*, 1998). Tobacco smoking is considered to be the most identifiable cause of adult death in the developed countries, with the exception of hypertension. In recent decades a growing body of literature has emerged, supporting the idea that smoking is associated with adverse effects on the kidneys. Evidence suggests that smoking has a detrimental effect on kidneys in diabetics and in individuals with hypertension and pre- existing renal disease. Smoking may also cause renal damage in healthy individuals, independent of other factors according to experimental studies and population based epidemiological studies. Smoking in diabetes has been linked to increased risks of microalbuminuria development, accelerated progression from microalbuminuria to manifest proteinuria and accelerated progression of manifest renal failure (Orth, 2004).

1.4.2.4 Protein Intake

More than sixty (60) years ago it was suggested that a low protein diet (LPD) could preserve renal function in patients with CKD (Addis, 1948). Addis hypothesized that a LPD would reduce the workload of surviving nephrons in diseased kidneys and thus minimizes further loss of renal function. Brenner *et al.,* (1982) extended this view and postulated the hyperfiltration theory based on animal studies. He suggested that sustained excesses of dietary protein cause increases in renal blood flow and glomerular filtration rate that lead to intrarenal hypertension, ultimately resulting in progressive sclerosis and deterioration of renal function (Brenner *et al*., 1982). Whether or not an excessive protein intake can be detrimental in subjects without kidney disease has not been thoroughly evaluated.

1.4.2.5 Obesity

Obesity, a component of the metabolic syndrome, has become a key worldwide problem. Although this phenomenon may result from altered dietary patterns and a sedentary lifestyle among people in affluent developed countries, it is now a rapidly emerging problem in developing countries. Worldwide obesity has increased 3-fold since 1980 and according to reports from the World Health Organization (W.H.O.), over one billion adults are overweight (body mass index [BMI] ≥25 kg/m^2) with at least 300 million being obese (BMI ≥ 30 kg/m^2) (WHO, 1998). There are further, great concerns about the rising prevalence of overweight and obesity among adolescents and children of school going age. Obesity contributes significantly to the burden of chronic diseases such as cardiovascular disease, cancers, type 2 diabetes, and hypertension, among others (O'Brien and Dixon, 2002).

The alarming increment of obesity worldwide has been paralleled by a steadily increasing incidence of ESKD as a result of type 2 diabetes and hypertension (SRAU, 2003; USRD, 2004). Unquestionably, much of the excess risk for CKD observed among people with obesity (El-Atat *et al*., 2003; Hall *et al*., 2004) is linked to the increased prevalence of hypertension and/or type II diabetes (El-Atat *et al*.,

2003; Mokdad *et al.*, 2003). However, it also appears that obese individuals diagnosed with hypertension and diabetes are at a higher risk of developing nephropathy, compared with leaner subjects with these conditions, independent of blood glucose concentration and other factors. In epidemiological studies, a high BMI was independently linked to proteinuria among diabetics (Savage *et al.*, 1995; Spangler and Konen, 1996), and hypertensive subjects (Ribstein *et al.*, 1995). Obesity may also aggravate existing nephropathies and is also associated with increased risk of graft failure after renal transplantation. Further evidence for a link between obesity and kidney damage is provided by the fact that weight loss in the obese reduces proteinuria and hyperfiltration (Chagnac *et al.*, 2003; Morales *et al.*, 2003).

1.4.2.6 Birth Weight

There are reports that low birth weight (LBW) is associated with increased risk of death from ischaemic heart disease (Barker, 1993). The elevated risk appears to be limited to those who had low birth weight as a result of growth retardation, rather than to those born prematurely. A considerable number of reports are now published relating intrauterine malnutrition to a number of diseases in adult life including cardiovascular disease (CVD), hypertension, diabetes and renal disease (Godfrey and Barker, 2000; Ingelfinger, 2004). The kidneys appear to be particularly sensitive to an unfavourable prenatal environment (Marchand and Langley-Evans, 2001; Ingelfinger, 2004). Studies of human fetuses and neonates have demonstrated an association between intrauterine growth retardation and nephron number and reduced renal volumes. Oligonephropathy or small number of nephrons may result in hyperfiltration and glomerular hypertension, which might lead to increased future risks of glomerulosclerosis, hypertension and renal failure (Brenner *et al.*, 1996). Two previous case controlled studies from the United States found that a birth weight below 2.5 kg was independently linked to an increased risk of ESKD among whites (Lackland *et al.*, 2001). Besides low birth weight, short stature has also been associated with unfavourable intra uterine

development. In cross sectional studies short stature has been linked with a degree of albuminuria in both diabetics and non diabetic. It has also been associated with an increased rate of nephropathy among type 1 diabetics (Jacobson, 1991).

1.4.2.7 Analgesics

More than fifty (50) years ago (Spuhler and Zollinger, 1953), observed an association between chronic interstitial nephritis and excessive consumption of combination analgesics containing phenacetin. Soon thereafter reports of an increased occurrence of renal papillary necrosis among heavy users of phenactin started appearing (Burry, 1966). This nephropathy associated with analgesic use was initially called phenacetin nephropathy. It was later renamed analgesic associated nephropathy (AAN) since a lot of analgesics came under suspicion of causing nephropathy. During the last two decades results from epidemiological studies have suggested that analgesics do not only cause classical AAN but may also increase the risk of CKD and ESKD in general. Moreover, there are indications that analgesic use may exacerbate pre-existing CKD. The mechanisms involved in analgesic- induced renal injury remains unclear, but cell injury, free radical formation, prostaglandin inhibition, reduced medullary blood flow and possibly an immunological mechanism are suggested modes of actions (Duggin, 1996; Gault and Barrett, 1998). Prostaglandin inhibition by aspirin and other NSAIDs causes redistribution of renal blood flow from the renal medulla to the renal cortex potentially resulting in the medullary ischaemia and eventual necrosis of the renal papillae (Sabatini, 1996).

1.4.2.8 Socio-Economic Status

It is evident that socio- economic status is linked to the development of ESKD since both low income and low educational level have been associated with elevated risk (Byrne *et al.*, 1994; Perneger *et al.*, 1995).

1.4.2.9 Occupational Exposures

Several occupational exposures have long been accused of impairing renal function and causing CKD (de Broe *et al.*, 1996). Exposure to organic solvents have predominantly been linked to the appearance and exacerbation of glomerulonephritis (Ravnskov, 2000). Previous literature has suggested an adverse renal effect from silica and several heavy metals such as cadmium, chromium and lead (Wedeen, 1997). The most persuasive evidence exists for cadmium; which has been known to cause proteinuria (Jarup, 2002) and has been linked to increased risk of ESKD (Hellstrom *et al.*, 2001).

1.4.2.10 Dyslipidaemia

Renal disease, in early as well as well as advanced stages, is associated with abnormalities in lipoprotein metabolism. Dyslipidaemia appears to be independently associated with increased progression rate of CKD in patients with kidney disease (Samuelsson *et al.*, 1997; Cusick *et al.*, 2004), and with increased risk of graft loss after renal transplantation (Castello, 2002). Moreover, there are indications that dyslipidaemia might initiate kidney disease. Two cohort studies in the general population reported links between elevated plasma triglycerides, high total serum cholesterol, and low high density lipoprotein cholesterol on the one hand and increases in serum creatinine at follow up on the other (Muntner *et al.*, 2000; Schaeffner *et al.*, 2003).

1.4.2.11 Genetic Susceptibility

There are indications that a generalized genetic susceptibility contributes to the development of ESKD (Buraczynska and Ksiazek, 2001; Freedman, 2003). The observation that there is a clear familial aggregation of ESKD due to diabetes, hypertension and glomerulonephritis, initiated the search for specific "candidate genes" that might be involved in renal diseases. It has been suggested that from various types of genetic association studies that genes of the renin angiotensinogen system and genes coding for cytokines and growth factors might be of interest,

among others (Buraczynska and Ksiazek, 2001). Some specific mutations are suggested to increase the susceptibility for glomerular damage, and are implicated in the aetiology of focal segmental nephrosclerosis (Winn, 2003). Studies using the genome scan approach, which has the potential for a more comprehensive evaluation of inheritance throughout the genome and to locate previously unknown genes related to diseases, have recently found evidence of susceptibility loci for diabetic nephropathy (Bowden *et al*., 2004).

1.4.2.12 Oxidative stress

Oxidative stress is defined as a disturbance in the equilibrium between antioxidants and prooxidants (reactive oxygen and nitrogen species or free radicals) with elevated levels of prooxidants resulting in possible tissue damage (Sies, 1991; Halliwell, 2007). Oxidative stress has been implied for a long time as a key process in the development of complications of diabetes mellitus and chronic kidney disease (Baynes, 1991; Stenvinkel, 2003).

Free radicals are identified by the presence of unpaired electrons in their outer orbitals that make them very unstable (Halliwell and Gutteridge, 1999). They are extremely reactive and have a very short half-life. Examples include superoxide ($O_2^{\bullet -}$), hydroxyl ($OH^{\bullet}$), peroxyle ($LOO^{\bullet}$), alkoxyl ($LO^{\bullet}$), hydroperoxyl ($HOO^{\bullet}$) nitric oxide ($NO^{\bullet}$), nitrogen dioxide ($NO_2^{\bullet}$) and peroxynitrite ($ONOO^{\bullet}$) radicals. However, substances like hydrogen peroxide (H_2O_2), hydrochlorous acid (HOCI), ozone (O_3), singlet oxygen ($^1O_2$) and hydroxy alkenals nitrous acid (HNO_2), dinitrogen trioxide (N_2O_3) and alkyl peroxynitrites (LOONO) lack unpaired electrons in their outer orbitals although they are highly reactive. Thus they are referred to as reactive oxygen/nitrogen species (ROS/RNS) (Halliwell and Gutteridge, 1984).

A pair of electrons on the outer orbital of molecular oxygen contributes to its stability. The formation of free radicals involves the activation of molecular oxygen

either through excitation as in the case of singlet oxygen or reduction when superoxide free radical hydrogen peroxide and hydroxyl free radical are concerned. Similarly, molecular scission of oxygen atom and the oxidation of molecular oxygen ion are means through which free radicals are formed through reduction.

1.4.2.12.1

Formation of Free radicals

Molecular oxygen can be reduced to form water in a four electron reaction like the one found in the mitochondria in the course of oxidation by cytochrome enzymes.

Superoxide radical is produced through a single-electron reduction of oxygen. Reactions of this nature take place through the excitation of solutions containing oxygen by ionizing radiation or ultrasound. Physiologically, free radicals can be formed through autoxidation of reducing sugars, non-enzymatic chemical reactions and enzymatic reactions like xanthine oxidase and NADPH-oxidase reactions. The superoxide radical has a short lifespan and becomes dismutated to form hydrogen peroxide in a reaction that may be spontaneous or that may be catalysed by the enzyme superoxide dismutase (SOD).

Two isoforms of SOD exist: cytosolic◎Cu/Zn-SOD and mitochondrial Mn-SOD. Both are regarded as antioxidant◎enzymes.

H_2O_2 produces an additional reactive substance such as hydroxyl radical. Hydroxyl radical is very reactive and has the shortest lifespan. It is formed from the breakdown of hydrogen peroxide through the Haber-Weiss and Fenton reactions. It causes direct damage to important biological macromolecules whilst it is reduced to water in these reactions. The catalase enzyme and peroxidases like glutathione peroxidase degrade H_2O_2 before it forms the highly reactive hydroxyl radical (Halliwell and Gutteridge, 1984; Gutteridge, 1994).

Fenton reaction

In this reaction ferrous iron catalyses the cleavage reaction of H_2O_2

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \cdot OH + OH^-$$

The Haber –Weiss Reaction

This reaction involves the splitting of H_2O_2 into more active $\cdot OH$ and OH^- free radical species (Gutteridge, 1994).

$$\cdot O_2^- + H_2O_2 \longrightarrow O_2 + \cdot OH + OH^-$$

Free radicals have the ability to attack biologically active molecules like lipids, nucleic acids, carbohydrates, proteins and amino acids due to their unique structure. Polyunsaturated fatty acids and membrane lipids are peroxidized by enzymatic and non enzymatic pathways. The end products of lipid peroxidation include cyclic endoperoxidases and lipid hydroperoxidases. These further produce smaller advanced glycation end products (ALE) like Malondialdehyde (MDA), glyoxal, methylglyoxal and acrolein (Miyata *et al.*, 1999; Maritim *et al.*, 2003).

1.4.2.12.2

Damage to nitric oxide and formation of peroxynitrite

Nitric oxides (NO), an endothelium dependent relax factor and a potent vasodilator is also capable of decreasing aggregation of platelet and proliferation of vascular smooth muscles. Therefore NO is provides protection against vascular remodeling as well as reducing blood pressure. NO is produced by an enzyme nitric oxide synthase of which there are three isoforms; inducible NOS (iNOS), constitutively expressed endothelial NOS (eNOS) and the neural NOS (nNOS) isoforms. The NOS enzyme splits the amino acid L-arginine to NO and citriulline

in a reaction that is dependent on NADPH. Low levels of NO have been reported in kidney diseases with explanations that it could be a mediator or an outcome of kidney damage (Wagner *et al.*, 2002). The NO may react with $\cdot O_2$ to form peroxynitrite (ONOO) whose effects are opposite to that of NO (Onozato *et al.*, 2002).

1.4.2.12.3

Antioxidants

An antioxidant significantly repairs, interrupts or slows down the oxidation of a substrate when available in reduced concentrations compared to that of an oxidant (Halliwell and Gutteridge, 1999). Antioxidants can originate from the body or be obtained diet. They are generally grouped into intracellular and extracellular antioxidants depending on the location (Rice-Evans and Burdon, 1993; Gutteridge, 1995). Intracellular antioxidants can further categorized into enzymatic and non-enzymatic antioxidants. Enzymatic antioxidants include SOD, catalase (CAT) that change possible oxidant substrates (superoxide anion radicals, hydroxyl radical and hydrogen peroxide) to less reactive types in the body (Gutteridge, 1995; Halliwell, 2007). The non-enzymatic cellular antioxidants are glutathione peroxidase (GPx) and glutathione reductase (GRx) (Er *et al.*, 2007; Halliwell, 2007). A number of extracellular antioxidants including proteins like transferrin, lactoferrin, albumin and cerruloplasmin; and uric acid inhibit the systemic formation of free radicals by segregating transitional metals by chelation in plasma. However, antioxidants like albumin, bilirubin and urate directly scavenge for free radicals and are thus referred to as scavenger antioxidants. Similarly a considerable amount of the ascorbic acid found in plasma has the ability to scavenge for peroxyl radical (Gutteridge, 1995; Halliwell and Gutteridge, 1999)

1.4.2.12.4

Oxidative stress and CKD

The rate of oxidative stress in CKD patients have been increasing over the years and this has been attributed to various causes. Early CKD is often associated with inflammatory processes which may play a role in oxidative stress usually measured as low GSH and high oxidized glutathione (GSSG) and increased lipid peroxidation (Turi *et al.*, 1997; Vas *et al.*, 2005). Low molecular weight substances like advanced glycation end products (AGE), proinflammatory cytokines (IL-1, IL-6, TNF-α) accumulate as GFR falls with the progression of CKD (Wittmann *et al.*, 2005). The interaction between the AGE-RAGE and cytokines stimulate inflammatory cells which results in respiratory burst, release of free radicals and chemoattractants. Collaboration between leukocytes and the surface of the dialysing membrane (partially bio incompatible) alongside possible bacterial contamination of the dialysing fluid likewise results in inflammation and subsequently oxidative stress (Locatelli *et al.*, 2003a; Stenvinkel *et al.*, 2005).

1.4.2.13 Metabolic syndrome

The metabolic syndrome (MetS) is defined as a constellation of risk factors of CVD and type 2 diabetes (Grundy *et al.*, 2004). It is mostly characterised by central obesity, dyslipidaemia (raised triglycerides and low high-density lipoprotein [HDL] cholesterol), hyperglycaemia and hypertension. The mets increases mortality (Thomas *et al.*, 2007) and it also associated with conditions like non alcoholc fatty liver disease (Abdeen *et al.*, 2006) and sleep apnoea (Vgontzas *et al.*, 2005), colorectal cancer and cancinoma of the breast and uterus (Chan *et al.*, 2007a; Xue and Michels, 2007). Quite recently it has also been linked with kidney dysfunction.

The incidence of MetS globally has reached epidemic levels. In the United Statess the prevalence of MetS is between 30-35% in both males and females and around 20-30% in the United Kingdom (Ford and Mannino, 2004; Tillin *et al.*, 2005; Cheung *et al.*, 2006). In Ghana, the prevalence of the MetS is about 14% as reported in a recent study by Owiredu *et al.,* (2011).

Age has been identified as a factor that affects the MetS causing an increase in the prevalence especially in people who are >60 years in the United States (Ford *et al.*, 2002). Possible culprits in the occurrence of this disorder include irregular timing of meals, urbanization, western lifestyle and westernization of diets.

A number of organizations including the World Health Organization (WHO), the US National Cholesterol Educational Program Adult Treatment Panel (NCEP ATP III), the European Group for the Study of Insulin Resistance (EGIR) and the International Diabetes Federation (IDF) have proposed definitions and sanctioned clinical criteria for the definition of MetS (Balkau and Charles, 1999; Alberti *et al.*, 2005). Altogether, the definitions and criteria give a catalogue with straightforward beneficial markers that are likely causes of cardiovascular disease such as dyslipidaemia, hypertension, obesity and diabetes. The NCEP ATP III criteria is the most commonly used and it has helped in the identification of components of the metabolic syndrome and has considered obesity as largely responsible for the increasing prevalence of the MetS (NCEP, 2001b; Grundy *et al.*, 2004). Whereas, insulin resistance as well as microalbuminuria are essential for the WHO criteria, upper body adiposity is vital for meeting the IDF criteria.

1.4.2.13.1

Pathophysiology of the metabolic syndrome

1.4.2.13.1.1

DYSLIPIDAEMIA AND CENTRAL OBESITY

Central obesity is initiated by a blend of genetic and environmental factors. In the pathophysiology of the metabolic syndrome a collection of hyperplastic and hypertrophic adipocytes play complex and important functions. Excessive production of triglycerides by the hepatocytes as a result of increased influx of free fatty acids in the liver results in hypertriglyceridemia. Furthermore, adipocytes produce inflammatory cytokines like IL-6, TNF-α and CRP with a reduction in the level of the anti-inflammatory cytokine adiponectin resulting in endothelial dysfunction (Sowers, 2007).

1.4.2.13.1.2

INSULIN RESISTANCE AND GLUCOSE INTOLERANCE

Reaven (1988) was the first to expound the impact of insulin resistance in the pathogenesis of the metabolic syndrome. Hyperinsulinaemia is present in insulin resistance in a bid to maintain euglycaemia. Furthermore, there is the failure to restrain the production of glucose by the liver and the kidneys as well as to regulate uptake of glucose by the adipose tissues and the muscles (Eckel *et al.*, 2005). In addition there is increased reabsorption of uric acid and sodium as a result of the hyperinsulinaemia resulting in hypertension and hyperuricaemia. This ultimately leads to diabetes.

1.4.2.13.1.3

HYPERTENSION

Nephrosclerosis due to hypertension may be blood pressure dependent or independent of blood pressure. Besides in hypertension and sodium retention secondary to obesity adipokines play a role because it stimulates sympathetic

activity in the kidneys (Zoccali, 2009). The RAAS is of utmost importance in the initiation of CKD and the obstruction of angiotensin II reduces oxidative stress and cytokines significantly because it affects glomerular haemodynamics and inflammatory mechanisms (Kurata *et al.*, 2006). Decreased GFR have been observed in patients with prehypertension with increased pressure load and proteinuria an indication that patients with mildly elevated blood pressure are at risk of renal injury.

1.4.2.13.2

Mechanism of renal disease in MetS

Inflammatory cytokines released due to insulin resistance result in the expansion of the glomerular mesangium, thickening of the basement membrane, podocytopathy and loss of integrity of slit pore diaphragm. Additional related causes include endothelial dysfunction, oxidative stress, activation of the renin angiotensin aldosterone system (RAAS) and elevated plasminogen-activator-inhibitor 1. This eventually results in glomerulosclerosis and tubulointerstitial injury (Sowers, 2007).

The pathology of kidney disease in the MetS has been demonstrated in animal studies (Kasiske *et al.*, 1992). Studies in obese zucker rats reported hyperphagia, obesity, hypertension, insulin resistance and dyslipidaemia due to a defect in the brain receptor in a scenario similar to MetS in humans. Hyperfiltration ensued and they finally had FSGS and glomerulogaly.

These findings have been confirmed in human subjects. GFR and RBF were elevated by about 50% 30% respectively in patients with severe obesity compared to lean controls as showed by Chagnac *et al.*,(2003).

A recent report has linked the MetS with a 6.9 fold rise in the odds ratio (OR) of glomerular hyperfiltration in healthy males with an average age 18 years

(Tomaszewski *et al.,* 2007). It is common knowledge that hyperfiltration results in proteinuria even in paients without diabetes. A graded incidence of microalbuminuria along with the number of components of the MetS has been reported by a couple of studies including that of Chen *et al.,* (2004) and Hao *et al.*,(2007). Focal segmented glomerulosclerosis (FSGS) has been established in subjects with severe obesity using kidney biopsy. Using multivariant analysis the prevalence of ESKD increased in subjects with high BMI after adjusments for existence of diabetes and BP (Iseki *et al.*, 2004; Hsu *et al.*, 2006). A study by Iseki *et al.*, (2004) on the occurrence of ESKD in a cohort of 100,000 subjects indicated that the number of subjects who developed ESKD increased in a graded manner after 17 years. Similarly the relative risk (RR) of developing ESKD increased significantly with increase in BMI in a cohort of 300,000 subjects (Hsu *et al.*, 2006). In conclusion Iseki and Hsu reported that elevated BMI was a strong and potentially variable risk factor for ESKD. Furthermore, both studies reported a synergistic relationship in the between the number of MetS components and the risk of CKD. Conclusions drawn from a cross-sectional study among Chinese subjects indicated that the MetS could be an essential risk factor for CKD (Chen *et al.*, 2007).

1.5 COMPLICATIONS OF CHRONIC KIDNEY DISEASE

Table 1.3 List of Complications of CKD

Congestive heart failure	Bone, joint, and muscle pain
Coronary artery disease	Changes in blood sugar
Hypertension	Peripheral Neuropathy
Pericarditis	Dementia
Stroke	Pleural Effusion
Hyperphosphataemia	Heart and blood vessel complications
Hyperkalaemia	Miscarriages and infertility
Secondary hyperparathyroidism	Seizures
Increased risk of infections	Anemia
Liver damage or failure	Bleeding from the stomach or intestines
Malnutrition	Hypermagnesaemia

Source: (Mitch, 2007)

1.5.1 Anaemia

Anemia is defined when there is a decrease in more than one of the major red blood cell (RBC) indices; hemoglobin concentration, haematocrit, or red blood cell count. Anaemia is defined by the WHO as a hemoglobin < 13 g/dl in men and post-menopausal women, < 12 g/dL in pre-menopausal women (WHO, 1968). The NKF defines anaemia as haemoglobin < 13.5 g/dL in men and < 12.0 g/dL in women (NKF/DOQI™, 2002). On the other hand, both NKF and European best practice guidelines advocate assessment of anaemia when haemoglobin level is below 11 g/dl and ponders recombinant human erythropoietin if haemoglobin is constantly < 11 g/dl to maintain target haemoglobin of > 11 g/dl (EBPG, 1999; KDOQI, 2006).

A normochromic, normocytic anaemia frequently accompanies progressive CKD (Besarab and Levin, 2000), and the general prevalence of CKD-associated anaemia is approximately 50% (McClellan *et al.*, 2004). Regardless of the stage at which anaemia is diagnosed in CKD; a strong correlation exists between between the

prevalence of anaemia and the severity of CKD. Twenty five percent (25%) of stage 1 CKD patients, fifty percent of those stratified to CKD stages 2, 3, and 4 and seventy five percent (75%) of CKD patients about to start dialysis reportedly have anaemia (McClellan *et al.*, 2004). While anaemia in CKD can result from various mechanisms (iron, folate, or vitamin B12 deficiency; blood loss due to-frequent blood sampling, haemodialysis and gastrointestinal bleeding; bone marrow suppression due to uraemic toxins and severe hyperparathyroidism, systemic/chronic inflammation, and shortened red blood cell survival; drugs-ACE inhibitors, angiotensin receptor blockers, theophylline; aluminium excess), decreased erythropoietin synthesis is the most important and specific aetiology causing CKD-associated anaemia. Erythropoietin, a glycoprotein, is secreted by the kidney interstitial fibroblasts (McClellan *et al.*, 2004) and is vital for the differentiation and growth of red blood cells in the bone marrow. In CKD, tubular atrophy produces tubulointerstitial fibrosis, which compromises renal erythropoietin synthetic capacity and results in anaemia. The anaemia of CKD increases morbidity and mortality from cardiovascular complications (angina, left ventricular hypertrophy (LVH) and worsening heart failure) (Besarab and Levin, 2000), which may result in further decline of kidney function and the establishment of a vicious cycle known as the "cardiorenal anaemia syndrome". The presence of LVH is linked with reduced survival rate of patients on dialysis. In reality, end stage kidney disease patients with LVH have lower survival rates than individuals without LVH (Levin *et al.*, 1996). Additionally, anemia is an independent cause of death in steady coronary artery disease (CAD) patients with CKD (Muzzarelli and Pfisterer, 2006).

The anaemia of CKD is treated via recombinant human erythropoietin (epo). This intervention has replaced transfusions as the basis of treatment and improved the survival of anaemic CKD patients (Fink *et al.*, 2001). The target level of haemoglobin in patients with CKD has varied as more findings have been reported. The major aim of treatment therefore is no longer to achieve normal

levels of haemoglobin since these target levels have been linked with increased mortality (Besarab *et al.*, 1998).

1.5.2 CKD-associated Mineral and Bone Disorders

The term "CKD-associated mineral and bone disorders" connotes bone and mineral metabolism abnormalities and/or extra-skeletal calcification secondary to the consequences of CKD (Moe *et al.*, 2006; Gal-Moscovici and Sprague, 2007). Renal osteodystrophy (ROD) is an array of histological changes, which arise in bone architecture of CKD patients. The primary site of phosphate excretion and 1-α-hydroxylation of vitamin D is the kidney. CKD patients develop hyperphosphataemia as a result of reduced 1, 25 dihydroxy-vitamin D levels that indicate decreased synthesis as a result of parenchymal scarring. Moreover, excretion of phosphate by the kidney is reduced. Consequently, serum calcium levels fall resulting in an increase in the rate of production of parathyroid hormone (secondary hyperparathyroidism). One prominent function of Parathyroid hormone is to increase phosphate excretion in the urine. In addition, it also increases plasma calcium levels by promoting bone resorption and increasing 1-α-hydroxylation of 25-hydroxy vitamin D produced in the liver (limited effect because of reduced kidney reserve from scarring). Rising phosphate levels are geneally observed in stage 3 CKD patients. Conversely, bone architecture is distorted quite early by secondary hyperparathyroidism just before serum phosphate level is noted to be abnormal. This gives an indication that treatment with phosphate binders should start when eGFR have declined below 50 mL/min per 1.73 m^2. A high or low bone turnover can result in changes in bone architecture. Four types of bone phenotypes ROD can be diagnosed in CKD patients namely osteitis fibrosa cystica (with high bone turnover due to secondary hyperparathyroidism), osteomalacia (resulting in low bone turnover and inadequate mineralization, often associated with reduced synthesis of vitamin D), adynamic bone disorder (with low bone turnover due to over-suppression of the

parathyroid glands), and lastly mixed osteodystrophy (with elements of both high and low bone turnover). The major type of ROD and CKD-mineral and bone disorder varies between pre-dialysis and end stage kidney disease patients. High bone turnover bone disease is most common in in pre-dialysis patients. Conversely, low bone turnover is common in dialysis patients. Majority of incidents of ROD is found in CKD patients with low turnover disease (Joy *et al.*, 2007). This predominant condition is due to the oversuppression of parathyroid hormone and high levels of calcium in the dialysis solutions (Hruska and Teitelbaum, 1995). The ability of phosphate retention to stifle the renal synthesis of 1, 25 dihydroxyvitamin D, acidosis and the lack of the physiologic inhibitory effect of vitamin D on parathormone secretion also contribute, albeit small, to the low turnover bone disease in CKD patients (Llach, 1995). CKD-associated mineral bone disorders significantly increase mortality in patients with CKD. In reality, hyperphosphatemia has been identified as the most significant risk factor associated with cardiovascular disease in CKD patients (Lee *et al.*, 2007). The precise mechanism underlying this relationship is stll unclear. It is believed to be related to hyperparathyroidism (El-Kishawi and El-Nahas, 2006) and vascular calcification due to elevated phosphate levels (Hutchison, 2007). The use of calcium based binders and excessive vitamin D therapy (Moe, 2006) influence vascular calcification and the associated cardiovascular mortality. Patients on haemodialysis with plasma phosphate level above the K/DOQI guideline objectives have a 40% higher rate of mortality compared to those having lower target levels (Noordzij et al., 2005). The main objective of therapy of CKD-associated bone and mineral disorders is to reduce phosphate levels (Coresh *et al.*, 2007). When phosphate or parathyroid levels begin to rise, the primary therapy is to restrict dietary phosphate intake. Serum phosphate concentrations should be maintained between 2.7 and 4.6 mg/dL among patients with CKD stages 3 and 4, and between 3.5 and 5.5 mg/dL for those with stage 5 CKD according to KDOQI guidelines. Various groups of phosphate binders can be applied to achieve this goal. For the treatment

of chronic conditions calcium-based formulations for the management of hyperphosphataemia due to CKD are the most widely used and have replaced aluminium binders since aluminum-associated toxicities have been established. However, calcium-based phosphate binders can induce hypercalcaemia, which increases the tissue calcium deposition, especially in the presence of hyperphosphatemia.

1.5.3 Cardiovascular Risk

It has been established that ESKD poses a great cardiovascular risk. The mortality rates as a result of the cardiovascular consequences is ten to hundred folds higher among patients on dialysis than individuals matched for age and sex in the general population (Foley *et al.*, 1998). The risk of cardiovascular disease as 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. Most of the traditional risk factors recorded in the general population increase the risk of cardiovascular disease in CKD patients. In reality, numerous Framingham risk factors are more prevalent among CKD patients as compared to those with normal kidney function. Additionally, non-traditional risk factors which are peculiar to CKD patients also contribute to the cardiovascular disease burden. Hypertension, a traditional cardiovascular risk factor, contributes to the cardiovascular risk connected to CKD. Investigations have shown that patients with hypertension are more susceptible to new or chronic cardiovascular events especially among individuals with CKD stage 2–3 (Muntner *et al.*, 2005). 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 a U-shaped relationship between systolic blood pressure and mortality in which fluctuating blood pressures are apparently associated with increased rate of mortality among stage 5 CKD patients. Rather than being aetiology for excess mortality, low systolic pressures may identify severely ill

group of patients. Recommendations of the KDOQI guidelines state a target blood pressure of <130/85 mm Hg for all kidney disease patients and <125/75 mmHg for patients with proteinuria exceeding 1g/24h.

Among patients with proteinuric (>1 gm/24 hr) progressive diabetic and nondiabetic kidney disease, the first–line drugs used are angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs). This is as a result of their kidney protective capabilities. Diabetes is associated with undesirable outcomes at every stage of CKD (Tonelli *et al.*, 2005). Besides, decreased fasting plasma glucose and /or glycated hemoglobin levels are linked with lower risk of all cause mortality and reduced cardiovascular death of marginal significance in patients with moderate to severe kidney impairment. The incidence of left ventricular hypertrophy (LVH), a complication which is elevated in relation to progressively lower levels of eGFR, is also a contributing factor of cardiovascular risk CKD patients. Hypertension and anaemia are two CKD related complications that are responsible for the occurence of LVH (Levin *et al.*, 1996). Investigators observed an independent risk of LVH in a prospective group of 2,423 patients with stage 3–4 CKD for the multifactorial endpoint of myocardial infarction and deadly coronary heart disease. Over a period of 102 months patients were followed. In multivariant analysis, LVH was linked with increased risk for composite and cardiac consequences hazard ratio (HR 1.67; 95% CI 1.34 to 2.07). Among stage 5 CKD patients, tobacco use is also linked with increased mortality and prevalence of heart failure (Combe *et al.*, 2004). Many CKD- associated cardiovascular risk factors are common in patients with this disease (non-traditional risk factors). Similarly, anaemia is risk factor for adverse cardiovascular cnsequences in CKD patients. Abberant levels of serum phosphate, calcium phosphate ion product, and parathyroid hormone, are independent cardiovascular risk factors in the situation of stage 5 CKD (Block *et al.*, 1998). Increased calcium–phosphate products and the cumulative dose of oral calcium-based phosphate binders compare with the extent and progression of arterial

calcification among dialysis patients (Goodman *et al.*, 2004) and those with stage 3 or 4 CKD. Serum phosphate levels were linked with high rates of death and myocardial infarction among patients with CKD stage 3 or 4 (Kestenbaum *et al.*, 2005; Kramer *et al.*, 2005). Consequently, arterial calcification result in clinical morbidity and mortality among the CKD population. Metabolic bone disease that is poorly managed leads to vascular calcification, which worsens arteriosclerosis and escalates vascular wall stiffness. One study of 96 patients, aged 18–70 with creatinine clearances ranging from 15–90 ml/min per 1.73 m^2, found coronary calcification in 64%, and 23% patients with severe calcification (Tomiyama *et al.*, 2006).

1.5.4 Dyslipidaemia

Dyslipidaemia is a major risk factor for cardiovascular morbidity and mortality and is common occurrence among patients with CKD. There is a wide variation in the lipid profiles in these patients. This gives an indication of the extent of kidney function and the amount of proteinuria (Kasiske, 1998). Generally, the prevalence of hyperlipidemia increases with declining kidney function, with the extent of hypertriglyceridaemia and rise of LDL cholesterol being proportional to the severity of kidney impairment. Several factors contribute to the development dyslipidaemia associated with chronic renal impairment. CKD patients have reduced activity of two key enzymes: hepatic triglyceride and lipase lipoprotein lipase. This interferes with uptake of triglyceride-rich, apolipoprotein B-containing lipoproteins by the liver and in peripheral tissue, resulting in the increased levels of these atherogenic lipoproteins in circulation. Hypercholesterolemia in nephrotic syndrome is often as a result of increased production and decreased breakdown of lipoproteins. The extent of lipoprotein abnormality is nearly proportional to the sum of proteinuria and inversely proportional to the level of serum albumin. Infusions of albumin or dextran or both, however normalize lipoprotein

concentrations, suggesting that oncotic pressure changes rather than hypoalbuminaemia stimulates increased lipoprotein production by the liver.

In-vitro experiments showing direct stimulation of increased hepatic apolipoprotein-B gene transcription within cells exposed to reduced oncotic pressure has been drawn from additional data supporting this hypothesis (Yamauchi *et al.*, 1992). Investigators have also intimated that hyperparathyroidism and the accumulation of calcium in pancreatic islet cells could possibly contribute to the dyslipidaemia associated with CKD (Arnadottir and Nilsson-Ehle, 1995). Clinical trials carried out in the general population have confirmed that the mortality rate of coronary heart disease is inversely proportional to LDL-cholesterol level reduction. Advantage of statins in lessening cardiovascular risk (i.e., composite outcomes) in CKD patients according to data is less conclusive. The largest clinical trial of statins was recently conducted in Germany among patients with ESKD (4D trial). Atorvastatin, in this study, did not to reduce death from, nonfatal myocardial infarction, fatal stroke, or nonfatal stroke in 200 diabetic patients with ESKD (Wanner *et al.*, 2005). The primary outcome of the relationship between total cholesterol levels and coronary heart disease (CHD) mortality has not been well elucidated. As a matter of fact, many observational studies of ESKD patients associate higher mortality rates with lower total cholesterol levels. For example, in a 10 -year prospective study, the significance of total cholesterol levels on mortality was studied in 1,167 ESKD patients (Iseki *et al.*, 2002). Hypercholesterolaemia (total cholesterol levels >200 mg/dl) was associated with high rate of mortality. More studies is required to establish whether or not low cholesterol recognizes a subgroup of more seroiusly ill patients or whether malnutrition/and or inflammation were confounding variables in these investigations. A comprehensive fasting lipid profile with measurement of total, LDL and HDL cholesterol and triglyceride levels should be part of the panel in the assessment of patients with CKD and dyslipidaemia. Subjects with increased cholesterol levels or other forms of dyslipidaemia other forms of dyslipidaemia

should be screened for secondary dyslipidemias before commencement of lipid lowering therapy (Eknoyan *et al.*, 2003). The guidelines of KDOQI recommend that all stages of CKD should be considered a CHD-risk equivalent. Patients with CKD are thus seen as being in the highest risk group for CHD and levels of LDL-cholesterol should be lowered below 100 mg/dl (2.6 mmol/l). Through the implementation of lifestyle changes CKD patients may realize LDL targets. Every adult with CKD should be screened for lipid abnormalities. The primary objective of therapy for CKD patients with nephrotic syndrome is to cause remission of the disease (Cleeman *et al.*, 2001). If this is not possible then any reduction in the amount of protein excreted in urine would be useful. Besides, nephrotic patients with high lipid levels have to be treated with a lipid lowering diet, which may help in reducing the level of total and LDL cholesterol. CKD patients have a higher burden of dyslipidaemia as comparde to the general population and are at greater risk for cardiovascular morbidity and mortality. This unbalanced cardiovascular disease burden puts CKD patients in the highest risk group, according to the treatment guidelines of the Adult Treatment Panel III (ATP III).

1.6 CLASSIFICATION AND STAGING OF CHRONIC KIDNEY DISEASE

The level of kidney function in all patients with chronic kidney disease can be uniformly measured regardless of the fundamental cause of the disease (Levey *et al.*, 2003). In the past, there has been a lack of agreement on how the progression of chronic kidney disease should be defined and classified. This may have contributed to under-diagnosis and under-treatment of early kidney disease resulting in lost opportunities for slowing or preventing disease progression (Pereira, 2000; NKF/DOQI™, 2002; Levey *et al.*, 2003; St Peter *et al.*, 2003). In the literature, it is widely agreed that starting treatment at the right stage in the progression of CKD is essential to help slow disease progression and prevent adverse outcomes (Pereira, 2000; NKF/DOQI™, 2002; Levey *et al.*, 2003; St Peter *et*

al., 2003). In an attempt to reach a consensus and provide a common ground on which to base future treatment and research, the American NKF/KDOQI work group developed a classification system that separated the period from very early kidney disease to ESKD into five stages (NKF/DOQI™, 2002). Definitions were based on renal function as measured by the GFR of the patient. Normal kidney function is said to equate to a glomerular filtration rate of 120-125 mm/min with deterioration in kidney function correlating with a reduction in the glomerular filtration rate. Table 1.1 describes the five stages of chronic kidney disease.

Stage 1 of CKD is described as the very early period of the disease where only minor kidney damage has occurred. Usually, clinical symptoms are absent at this point, which make diagnosis very difficult. This is the ideal time to provide treatment for the underlying kidney disease, along with appropriate management of allied conditions like hypertension and diabetes.

Patients who are classified as having Stage 2 CKD have a glomerular filtration rate of between 60 and 89 ml/min/ 1.73 m^2 and suffer from a mild degree of kidney damage. Aggressive management of the underlying causes of the disease and emerging manifestations, for example, calcium and phosphate imbalance, hyperglycaemia and anaemia, are recommended (Silverberg, 2003; St Peter *et al.*, 2003).

Stage 3 CKD indicates a further decline in kidney function with possibly some clinical signs beginning to appear. As mentioned previously, it is not uncommon for a patient to reach this stage of the disease without knowing that they have a problem. Again ongoing specialist treatment and follow up of these patients is essential to try and maintain kidney function and prevent such complications as cardiovascular disease, anaemia, malnutrition and bone disease.

Stage 4 of CKD means that end stage failure is imminent and preparation for renal replacement therapy (dialysis or transplantation) is required.

Stage 5 CKD is defined as ESKD where dialysis or transplantation is mandatory to sustain life.

The need to provide a provide a common language for communication among providers, patients and their families, investigators and policy-makers was the reason the American National Kidney Foundation developed the five-stage classification system. Defining chronic kidney disease this way provides opportunities to direct the most effective treatment at a particular stage of the disease process (Compton *et al.*, 2002).

In addition, classification seeks to provide a framework for developing guidelines for clinical practice, clinical performance measures, and improvement of continuous quality tasks (Parker *et al.*, 2004). The classification of the stages of kidney disease by the American National Kidney Foundation's has been integrated in some recent American and British literature in association with policies for prevention and early discovery of CKD (Compton *et al.*, 2002; Parmar, 2002; St Peter *et al.*, 2003). As this classification system has only been available for almost a decade it is difficult to predict the extent to which it will be utilized internationally. Kidney function and the outcome of kidney disease have been outlined along with a way of defining the loss of kidney function into stages. The five stages of chronic kidney disease, as described were developed in an attempt to provide a common language for nephrology health care professionals to use to promote international best practice in the management of CKD.

1.6.1 GLOMERULAR FILTRATION RATE

Glomerular filtration rate (GFR) is defined as the rate at which filtered fluid flows through the kidney. Creatinine clearance (C_{Cr} or CrCl) refers to the amount of blood plasma cleared of creatinine per unit time and is a convenient measure for estimating the GFR. Together, GFR and C_{Cr} may well be accurately calculated by

relative measurements of substances in the blood and urine, or calculated by formulas using only a blood test result (eGFR and eC_{Cr}). These test results are important in the assessment of the excretory capabilities of the kidney. For example, classification of CKD and dosage of drugs that are excreted mostly in urine are based on GFR (or creatinine clearance). Various methods of estimating GFR are briefly described below:

1.6.1.1 Clearance method:

The idea behind renal clearance was proposed as a way of expressing the relationship between the excretion per unit time and the concentration in the plasma which is obviously an index of the kidney's ability to clear the blood of any substance (Harvey, 1980). Measurements of GFR are by tradition based on the renal clearance of a plasma marker, expressed as the volume of plasma wholly cleared of the marker per unit time. If the marker has no extra-renal elimination, tubular reabsorption or secretion then the clearance is defined by the formula

GFR = UV/P, where

U = Urinary Concentration of the substance

V = Urine flow rate (urinary volume/time)

P = Average plasma concentration

The perfect marker should be endogenous; in addition it must be filtered freely by glomerulus. Futhermore it should neither be reabsorbed nor secreted by the renal tubule and eliminated solely by the kidney. Such a marker is not yet recognized. A variety of markers used to measure GFR include exogenous (inulin, iothalamate) or endogenous (urea, creatinine) substances.

A) Exogenous Substances

i) Inulin: a polymer of fructose with a molecular weight of 5200 daltons is regarded as the gold standard for the estimation of GFR. It is filtered freely by glomerulus, and is neither secreted nor reabsorbed by the kidney tubules.

Metabolically, it is inert and excreted only by the kidney. It needs constant intravenous infusion to keep up plasma level and once balanced state has been achieved, plasma and timed urine specimen levels of the marker are measured. However, analysis of inulin is technically challenging, time consuming, labour intensive, expensive and unsuitable for outpatient use (Smith, 1951).

ii) Non-radiolabelled contrast media: - Besides inulin, non-radiolabelled contrast media infusion (iothalamate/iohexol) have also been used to measure GFR. The ability to perform urography and estimation of GFR in a single examination is a major advantage (Brown and O'Reilly, 1991). Cumbersome measurement makes it inappropriate for day to day clinical practice.

iii) Radiolabelled compounds: - A number of radiolabelled substances have been used to measure the GFR in humans, as very small non-poisonous amounts of the compound can be used and can be measured using conservative counters even at very low concentrations. Amongst these is [^{51}Cr] EDTA, [^{125}I] iothalamate, [^{99}Tcm] DTPA, [^{131}I] Hippuran to mention a few (Donker *et al.*, 1977; Apperloo *et al.*, 1996). Disadvantages are that some radiation is administered, radiopharmaceuticals are more costly, Gamma camera and skilled personnel are required. Therefore it's impossible to use the chelates for the routine assessment of GFR.

B) Endogenous Substances

i) Urea (MW 60 dalton) was one of the first markers for assessing GFR (Chasis and Smith, 1938) however presently is not used in clinical practice due to numerous reasons. Urea production is erratic and changes with protein intake. Readily it is reabsorbed by tubules and again amount of reabsorption is erratic. Hydration status of the individual also affects urea clearance clearly; in patients with depleted intravascular volume, high plasma levels accompany decreased urine flow. Also many substances may interfere with its estimation.

ii) Creatinine (M.W 113 daltons) is produced through nonenzymatic dehydration of creatine and phosphocreatine. Muscle mass therefore is the main determinant (98%) of the creatinine pool. Dietary consumption of meat is another source of creatinine. Endogenous creatinine clearance which gives as an estimate of GFR was first proposed by Popper and Mandal in 1937 (Popper and Mandel, 1937) and is still highly patronized in clinical practice. However, its performance and interpretation present alarming difficulties: Changes in the rate of production of creatinine, accurate measurement of plasma creatinine, some level of secretion by the renal tubules and the difficulty of obtaining complete, accurately timed urine specimens (Payne, 1986; Spencer, 1986).

Creatinine is generally measured by the Jaffë colorimetric reaction using over the past century, using alkaline picrate with which it forms an orange red complex. Numerous substances such as ascorbic acid, uric acid, ketones and ketoacids, plasma proteins, bilirubin, fatty acids, urea, cephalosporins and glucose interfere with Jaffë's colorimetric assay for estimation of plasma creatinine resulting in erroneously high values.

Furthermore, tubular secretion and induction of true elevation of plasma creatinine is inhibited by drugs such as triametrine, spironolactone, amiloride, probenecid, cimetidine, trimethoprim and high dose salicylates or pyrimethamine (Gerard and Khayam-Bashi, 1985; Weber and van Zanten, 1991). Enzyme based assays have enhanced precision comparable to high performance liquid chromatographic techniques because they lack this interference (Gerard and Khayam-Bashi, 1985). As a result of tubular secretion creatinine clearance (Ccr) usually overestimates GFR. This represents 10-40% of GFR in normal renal function with clear interindividual variability. In patients with decreased kidney function tubular secretion can increase to above 100% especially among those with glomerulopathy and proteinuria (Shemesh *et al.*, 1985).

1.6.1.2 GFR prediction from plasma creatinine.

An estimate of bed side GFR is often obtained from plasma creatinine concentration alone in clinical practice though with some level of accuracy (Perrone *et al.*, 1992). A formula that will permit an immediate estimation of GFR from plasma creatinine has been considered by a number of researchers. Approximation of GFR from plasma creatinine may give erratic results because plasma creatinine is dependent on GFR as well as on muscle mass which varies with gender, agen and weight. Cirrhosis and muscle wasting diseases lead to a reduction in plasma creatinine; conversely ingestion of high amounts of protein can result in increase in plasma creatinine levels of up to 10% (Hull *et al.*, 1981). Furthermore, a marked reduction in GFR can be present before it shows in the concentration of plasma creatinine beyond the upper limit of the refernce range. The value of these formulae for GFR prediction is likely to increase when there is an accurate plasma creatinine measurement in addition to inhibition of tubular secretion of creatinine by cimetidine. To improve the estimation of GFR from plasma creatinine concentration, formulae have been derived which incorporate variables like weight, height, age, and gender.

1.6.1.3 GFR estimation by new endogenous markers:-

a) β2-Microglobulin (M.W 11815 dalton) is filtered at glomerulus like water. 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. The estimation of this substance entails the use of expensive radioimmunoassays and this has limited its use in clinical practice. 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).

b) Cystatin C is a 13-KD protease inhibitor which is produced generally by nucleated cells. It is neither affected by the muscle mass nor sex of an individual. Its production, unlike β2-microglobulin is not affected by states of inflammation or malignant conditions. Cystatin C is usually excreted by filtration through the glomerulus and metabolized in the cells of the proximal tubules. Its measurement has been projected as an alternative and more precise marker of GFR compared to creatinine especially among patients with slight to moderate reductions in GFR (Grubb *et al*., 1985; Newman *et al*., 1995).

1.6.2 Measurement of GFR using predictive equations

Multiple formulae exist to accurately estimate kidney function by correcting for factors such as variations in muscle mass in men versus women or in African American versus white people and changes in muscle mass due to aging. The most commonly used equations are the Cockroft-Gault (CG) equation (Cockcroft and Gault, 1976) and the 4v and 6v Modification of Diet in Renal Disease (MDRD) (Levey *et al*., 1999a) equations. Recently, the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) formula, (published in May 2009), has been added. This was developed in a bid to create a formula more detailed compared to the MDRD formula, particularly when real GFR is > 60 mL/min per 1.73 m^2 (Levey *et al*., 2009). Rule *et al*., (2006) have maintained that, because these formulae are derived from patients with kidney disease, they may not predict kidney function in patients without kidney disease. Mostly, clinicians use the MDRD equation because of its accessibility on the internet, where one can simply insert in values for age, weight, race, and sex to give an estimated GFR. It should be noted that all these formulae have large confidence intervals such that insignificant changes in actual GFR are difficult to distingish by this method.

1.7 DIAGNOSIS OF CKD

The first step in the diagnosis of CKD in a patient with elevated creatinine is to categorize the patient's presentation as one of three possible forms of kidney disease: post renal failure, pre renal azotemia, or intrinsic kidney damage.

Obstructive uropathy is the most common type of post renal failure. Intrinsic obstruction of urinary flow leads to obstructive uropathy (eg, stone, tumor, blood clot, or papillary necrosis) or by outward obstruction (eg. prostatic hypertrophy, retroperitoneal fibrosis, retroperitoneal tumor [lymphoma or metastatic disease]). The second and much less common form of post renal failure is renal vein thrombosis (Wysokinski *et al*., 2008).

Pre-renal azotemia, another of the reversible forms of CKD, results from a decrease in blood flow to the kidney that results in kidney dysfunction and an elevated serum creatinine level. It is often characterized by events such as thrombosis, acute renal artery embolism and dissection. A pre-renal component presents as an additional increase in serum creatinine levels from preceding reference values especially in many CKD patients. History and physical examination are critical for identifying pre-renal azotemia. The physician should look out for signs like nausea, vomiting, diarrhea, increased dosage or fresh usage diuretics, unforeseen resolution of long-standing oedema, weight loss, or orthostatic symptoms in the patient's history. In the course of examination, recumbent and upright blood pressure and pulse are the most important tools for evaluating extracellular volume depletion. Disease conditions like chronic heart failure, liver disease, and the nephrotic syndrome usually have the appearance of volume overload (eg, oedema, rales, abdominal fluid wave); however the kidney behaves as if there were dehydration, which results in a possible increase in the level of serum creatinine.

A number of markers of kidney damage mostly prominently the fractional excretion of sodium (FENa), helps in recognizing patients with oliguric pre-renal azotemia (Kohli *et al*., 2006). Other laboratory findings that may aid in the

diagnosis of pre-renal azotaemia include elevated levels of serum uric acid and serum calcium and an increase in the blood urea nitrogen (BUN) to serum creatinine ratio to above 20:1 (Morgan *et al.*, 1977).

Thirdly, the final source of an elevated serum creatinine level includes disease of the kidney tissue itself. There are basically three types of tissue in the kidney: glomerular tissue (primary glomerular disease; secondary glomerular diseases attributed to other conditions [eg, systemic vasculitis, diabetes, hypertension, amyloidosis]); vascular tissue, which may be affected by systemic vasculitis, atheroemboli, and thromboemboli; and interstitial tissues, which can be affected by sickle cell anaemia, abuse of analgesics, and certain medications (eg, antibiotics, proton pump inhibitors, NSAIDs).

Urine microscopy, provides valuable information on condition that the urine sample is fresh (<20 minutes after voiding). The presence of more than 25 dysmorphic RBCs is a common surrogate for RBC casts. RBCs will have a distorted appearance because they have traversed the glomerular basement membrane to reach the urinary space (glomerulonephritis). In contrast, RBCs entering the urine from other parts of the urinary tract will not be distorted and so will be unimorphic. Although the use of this surrogate is accepted in practice, careful evaluation shows that dysmorphic RBCs are no more suggestive of glomerulonephritis than is plain haematuria in the presence of substantial proteinuria (Pollock *et al.*, 1989; Ward *et al.*, 1998).

1.7.1 24-Hour Urinary Protein Excretion Test.

24-hour urinary protein excretion in patients with glomerular disease (eg, nephrotic syndrome) is defined, as urinary protein excretion of at least 3.5 g/d per 1.73 m^2 but can be much higher. Even though considerable proteinuria (1-5 g/d per 1.73 m^2) is mostly attributed to vasculitis, proteinuria is more marked in primarily glomerular forms of kidney disease. Interstitial kidney disease usually

presents with little or no proteinuria; however, up to 2 g of urinary protein, primarily tubular or Tamm-Horsfall protein, may be excreted daily (Graves, 2008).

1.7.2 Hypertension

Hypertension, especially when it occurs early in the course of renal failure, can be a useful tool in evaluating intrinsic forms of kidney disease. Most patients with vasculitis of the kidney will have hypertension which is often severe. Among patients with glomerulonephritis, hypertension is less likely to develop early in the course of the disease compared to patients with vasculitis. In patients with interstitial forms of kidney disease, hypertension only develops as they near end stage kidney disease (Graves, 2008).

1.7.3 Time Course of Increase in Serum Creatinine Level.

When available, the time course of the increase in the serum creatinine level is very helpful in identifying the type of renal disease causing its elevation. When left untreated, vasculitis of the kidney and diseases such as Goodpasture syndrome, Wegener granulomatosis, and lupus vasculitis rapidly progress to renal failure, reaching end stage or requiring dialysis support within weeks or months of the beginning of the disease. Although untreated glomerulonephritis may have a rapid course, kidney disease usually develops more slowly, with low levels of GFR reached in a period of 2 to 10 years. Interstitial renal disease has a more indolent course, reaching low levels of GFR only after 10 to 20 years. However, a rapidly increasing serum creatinine level is possible with allergic interstitial nephritis and acute tubular necrosis (Graves, 2008).

1.7.4 Radiography.

Renal ultrasonography with arterial Doppler studies is the single most important test for evaluating all patients with an elevated creatinine level. First and most importantly, it is the least invasive method for identifying obstructive uropathy,

the most reversible form of renal failure. Second, it provides information on renal size. If the kidneys are smaller than 7 to 8 cm, then the likelihood of a reversible form of kidney disease is extremely low. Large kidneys (>12- 13 cm) have a specific differential diagnosis, including reversible conditions like acute glomerulonephritis, infiltrative diseases of the kidney (leukaemia, lymphoma, Hodgkin disease, multiple myeloma, and amyloidosis), and permanent conditions such as diabetic nephropathy, polycystic kidney disease, and obstruction. The Doppler part helps in the identification of patients with bilateral renal artery stenosis, whose kidney function would profit from effective angioplasty (Graves, 2008).

1.8 AIMS AND OBJECTIVES

The incidence of CKD and thus ESRD is consistently increasing at a rate of 6% per annum worldwide. This rate is much higher than the rate at which the world population is growing, which is estimated at 1.2% yearly (Bamgboye, 2006). Sub-Saharan African countries contribute 5% of the total world CKD population. In Ghana data on the prevalence of CKD has been varied over the years. Bamgboye (2006) put the prevalence of ESRD in Ghana per million people at 1.6%. Addo *et al.*, (2009) put the prevalence of CKD among Ghanaian hypertensive patients at 4%. Renal replacement therapy (mainly haemodialysis and peritoneal dialysis) is available only in the two teaching hospitals, and the estimated cost of dialysis is GHC 57,600 (approximately $44,300) per patient per annum. This amount is rather high for a country with a per capita of $1500 and a GDP of 6.3%. The first renal transplant in this country was performed at the end of 2008 by a team of Ghanaian and British surgeons. Moreover, the number of patients requiring renal replacement therapy is increasing globally, by up to 7% annually according to some reports (Gansevoort *et al.*, 2004; Jones *et al.*, 2005).

The overall aim of this study was to evaluate the use of renal function equations in the assessment of renal function in CKD and to identify specific oxidative and metabolic risk factors in CKD.

The specific objectives are as follows:

- To examine the applicability of seven predictive equations in the estimation of GFR for the stratification of CKD.

- To explore the association between the MS and the risk of CKD among Ghanaian patients presenting with CKD.

- To determine the prevalence of anaemia among subjects with CKD and identify the cardiovascular risk markers among subjects with anaemia.

- To assess the lipid profile and oxidative stress/lipid peroxidation in patients presenting with CKD by determining relevant oxidative stress markers (MDA) and antioxidant levels (vitamins A and C, catalase and uric acid).

- To investigate the electrolyte and electrolyte ratios and their relationship with parathyroid hormone (PTH) among CKD patients.

Chapter 2

MATERIALS AND METHODS

2.1 RECRUITMENT OF SUBJECTS

This study was conducted between August 2008 and September 2009 among 146 patients with various chronic kidney diseases. To qualify for recruitment, subject must be diagnosed of a specific kidney disease and or about to start renal replacement therapy (haemodialysis or peritoneal dialysis). 50 patients were randomly recruited from the medical unit and the diabetic clinic of the Tamale Teaching Hospital in the Northern Region of Ghana and the remaining 96 from the dialysis centre (yet to start dialysis) and diabetic clinic of the Komfo Anokye Teaching hospital in the Ashanti region of Ghana for this study. The aetiology of the CKD ranged from diabetic nephropathy, 90 (61.6%); chronic glomerulonephritis, 12 (8.2%); adult polycystic kidney disease, 1 (0.7%); hypertensive nephropathy, 10 (6.8%) and chronic kidney disease with unknown aetiology, 33 (22.6%). Another eighty (80) healthy subjects were studied as controls. The participation of the respondents who are all indigenes of Ghana was voluntary and informed consent was obtained from each of them. The study was approved by the Committees on Human Research Publication and Ethics.

2.2 MEASUREMENT OF ANTHROPOMETRIC VARIABLES

Anthropometric measurements included height to the nearest centimeter without shoes and weight to the nearest 0.1 kg in light clothing. Subjects were weighed on a bathroom scale (Zhongshan Camry Electronic Co. Ltd, Guangdong, China) and their height measured with a wall-mounted ruler. Body mass index (BMI) was calculated by dividing weight (kg) by height squared (m^2). Waist circumference (to the nearest centimeter) was measured with a Gulick II spring-loaded measuring tape (Gay Mills, WI) midway between the inferior angle of the ribs and the suprailiac crest.

2.2.1 Blood Pressure (using Krotkoff 1 and 5)

Blood pressure was measured by trained personnel using a mercury sphygmomanometer and a stethoscope. Measurements were taken from the left upper arm after subjects had been sitting for >5 min in accordance with the recommendations of the American Heart Association (Kirkendall *et al*., 1967). Duplicate measurements were taken with a 5 minute rest interval between measurements and the mean value was recorded to the nearest 2.0 mmHg.

2.3 URINALYSIS

Early morning urine was collected in plastic containers from the respondents and urine protein was determined using the dip-stick qualitative method (CYBOW™ DFI Co Ltd, Gimhae-City, Republic of Korea).

Principle

The test is based on the protein error of indicators principle. When pH is held constant by a buffer indicator, dyes release H^+ ions because of the protein present and change colour from yellow to blue-green.

2.4 SAMPLE COLLECTION AND PREPARATION

Venous blood samples were collected after an overnight fast (12 – 16 hours). About 7 mls of venous blood were collected and, 5 ml dispensed into vacutainer® plain tubes. After clotting, it was then centrifuged at 500 g for 15 min. The serum was stored at - 80°C until assayed. The remaining 2 ml were dispensed into tubes containing 2.5 µg of dipotassium ethylenediaminetetraacetic acid (K_2 EDTA) as an anticoagulant.

2.4.1 Biochemical assays

Serum biochemistry was performed on the ATAC 8000 Random Access Chemistry System (Elan Diagnostics, Smithfield, RI, USA). Parameters that were determined include: liver function tests - total-protein (T-PROT), albumin (ALB) and globulin;

renal function tests – serum sodium (Na^+), serum potassium (K^+), blood urea nitrogen (BUN), serum creatinine (CRT), serum uric acid; electrolytes - serum calcium (Ca^{2+}), serum magnesium (Mg^{2+}) and serum phosphate (PO_4^{3-}). Adjusted calcium was calculated from the formula: Adjusted calcium (mmol/l) = total calcium (mmol/l) + 0.02 × [40 – serum albumin (g/dl)]. Also lipid profile which include total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C), very low density lipoprotein cholesterol (VLDL-C), low density lipoprotein cholesterol (LDL-C) and coronary risk were determined. The methods adopted by the automated instrument for the determination of the above parameters are as follows and all reagents are from JAS™ diagnostics, Inc. (JAS Diagnostics, Inc. Miami Florida, USA).

2.4.2 Albumin (BCG)

Principle and Method

At a controlled pH, bromocresol green (BCG) forms a coloured complex with albumin. The intensity of colour at 630 nm is directly proportional to albumin content. The instantaneous initial absorbance is obtained as suggested by Webster (1977). The method used by the JAS™ albumin reagent is based on that of Doumas *et al.,* (1971).

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

2.4.3 Total Protein (Biuret)

Principle and Method

The present method is based on the modification of method 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 protein present when compared to a solution with known protein concentration.

$$Protein + Cu^{2+} \xrightarrow{Alkali} Colored\ Complex$$

2.4.4 Cholesterol

Principle and Method

The present method utilizes a phenol substitute (4-aminoantipyrine (4-AAP) that performs like phenol but without being corrosive. The intensity of the red colour produced is directly proportional to the total cholesterol in the sample when read at 500 nm.

$$Cholesterol\ Esters \xrightarrow{Cholesterol\ Esterase} Cholesterol + Fatty\ Acids$$

$$Cholesterol + O_2 \xrightarrow{Cholesterol\ oxidase} Cholest-4-en-3-one + H_2O$$

$$2H_2O_2 + HBA + 4AAP \xrightarrow{Peroxidase} Quinoneimine\ (red\ dye) + 4H_2O$$

2.4.5 Triglycerides

Principle and Method

The present method uses a modified Trinder (Trinder, 1969; Barham and Trinder, 1972) colour reaction to produce a fast, linear, endpoint reaction (Fossati and Prencipe, 1982; McGowan *et al.*, 1983). Triglycerides in the sample are hydrolyzed by lipase to glycerol and fatty acids. The glycerol is then phosphorylated by ATP to glycerol-3-phosphate (G3P) and ADP in a reaction catalyzed by glycerol kinase. G3P is then converted to dihydroxyacetone phosphate (DAP) and hydrogen peroxide by glycerophosphate oxidase (GPO). The hydrogen peroxide then reacts with 4-aminoantipyrine (4-AAP) and 3, 5-dichloro-2-hydroxybenzen (3,5-DHBS) in a reaction catalyzed by peroxidase to yield a red coloured quinoneimine dye. The intensity of the colour produced is directly proportional to the concentration of triglycerides in the sample.

$$Triglycerides + H_2O \xrightarrow{Lipase} Glycerol + Fatty\ Acids$$

$$Glycerol + ATP \xrightarrow{Glycerol\ kinase} G3P + ADP$$

$$G3P + O_2 \xrightarrow{Glycerolphosphate\ oxidase} DAP + H_2O_2$$

$$H_2O_2 + 4AAP + 3,5 - DHBS \xrightarrow{Peroxidase} Quinoneimine\ (red\ dye) + 2H_2O$$

2.4.6 HDL-Cholesterol

Principle and Method

The method employed herein is in a two reagent format. The first reagent contains anti human β-lipoprotein antibody which bind to lipoproteins (LDL, VLDL and chylomicrones) other than HDL. The second reagent contains enzymes which then selectively react with the cholesterol present in the HDL particles. Consequently only HDL cholesterol is subject to cholesterol measurement. The primary reading is done at 600 nm and the secondary at 700 nm.

2.4.7 Urea Nitrogen (BUN)

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

Principle and Method

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 to yield glutamate. An equimolar quantity of NADH undergoes oxidation during the reaction catalyzed by Glutamate dehydrogenase (GLDH) resulting in a decrease in absorbance (340 nm) that is directly proportional to the urea nitrogen concentration in the sample.

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

$$NH_3 + \alpha - Ketoglutarate + NADH \xrightarrow{GLDH} L - Glutamate + NAD^+$$

2.4.8 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.

Principle and Method

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 ingredients 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.

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

2.4.9 Uric Acid

Uric Acid measurements are most commonly used in the diagnosis of gout. Increased levels (hyperuricaemia) may be observed in leukemia, polycythaemia, atherosclerosis, diabetes, hypothyroidism, and conditions associated with decreased renal function.

Principle and Method

The JAS™ procedure uses uricase, peroxidase and the chromogen TBHB to yield a colorimetric end product. Uric acid is oxidized by Uricase to allantoin and hydrogen peroxide. TBHB + 4-aminoantipyrine + hydrogen peroxide, in the presence of peroxidase, produce a quinoneimine dye that is measured at 520 nm.

The colour intensity at 520 nm is proportional to the concentration of Uric Acid in the sample.

$$Uric\ Acid + O_2 + H_2O \xrightarrow{Uricase} Allantoin + CO_2 + H_2O_2$$

$$H_2O_2 + 4-Aminoantipyrine + TBHB \xrightarrow{Peroxidase} Quinoneimine + H_2O$$

2.4.10 Magnesium

Magnesium in the body is found primarily in the bone with some in soft tissues, blood cells, and serum. Decreased levels have been observed in cases of diabetes, alcoholism, diuretics, hypothyroidism malabsorption, hyperalimenation, myocardial infarction, congestive heart failure and liver cirrhosis. Increased serum levels have been found in renal failure, diabetic acidosis, Addison's disease and vitamin D intoxication.

Principle and Method

More recently, colorimetric dye complexing methods have been developed and are in popular use. These procedures use such dyes as calmagite, eriochrome lack T, magon, and methylthymol blue (Tietz, 1994). The JAS magnesium uses an arsenazo dye which binds preferentially with magnesium. The absorbance of the arsenazo magnesium complex is measured at 570 nm and is proportional to the concentration of magnesium present in the sample. Calcium interference is prevented by incorporation of an unconventional calcium chelating agent.

2.4.11 Calcium

Increased serum calcium may be observed in hyperthyroidism, vitamin D detoxification multiple myeloma and some neoplastic diseases of bone. Decreased serum calcium may be observed in hypoparathyroidism, vitamin D deficiency, steatorrhoea, nephrosis, and nephritis (Tietz, 1994).

Principle and Method

The present procedure uses arsenazo III and has been mixed to provide a highly sensitive and stable reagent system. The reagent is provided as a convenient ready to use liquid. Calcium reacts with arsenazo III in a slightly alkaline medium to form a purple-coloured complex which absorbs at 650 nm. The intensity of the colour is proportional to the calcium concentration.

$$Calcium + Arsenazo\ III \xrightarrow{Alkaline\ Medium} Calcium - Arsenazo\ Complex\ (purple\ colour)$$

2.4.12 Phosphorus

Calcium and phosphate in serum usually exhibit a reciprocal relationship. An increase in one of these components is usually accompanied by a decrease in the other. Increased serum phosphorus levels may be found in hypervitaminosis, hypoparathyroidism and renal failure. Decreased serum phosphorus levels may be found in rickets, hyperparathyroidism, and the Fanconi syndrome, which is associated with a defect in reabsorption of phosphorus from the glomerular filtrate (Tietz, 1994).

Principle of the method

Phosphorus in serum reacts with ammonium molybdate to form phosphomolybdate, which is then reduced by stannous chloride and hydrazine sulphate to molybdenum blue (Amador and Urban, 1972). The intensity of the colour is measured at 640 nm.

$$Inroganic\ Phosphorus + H_2SO_4 + Ammonium + Molybdate \xrightarrow{Acidic\ Medium} Unreduced\ Phosphomolybdate\ Complex$$

2.5 HORMONAL ASSAY

Serum intact PTH was measured by immunoenzymatic assay, a solid phase Enzyme Amplified Sensitivity Immunoassay performed on microtitre plates (Genway Biotech Incorporated, Cat. No.: 40-056-205022).

2.5.1 Biological Activities

Human parathyroid hormone (hPTH) is a major physiological regulator of phosphocalcic metabolism. hPTH increases serum calcium concentration by its actions on kidney (enhancing tubular Ca^{2+} reabsorption and phosphate excretion) and bone (Stimulating osteoclastic activity and bone (stimulating osteoclastic activity and bone resorption). It indirectly affects intestinal absorption of Ca^{2+} by stimulating renal 1α–hydroxylation of 25 hydroxyvitamin D. The release of PTH is controlled in a negative feedback loop by the serum concentration of Ca^{2+}. PTH is synthesized in the chief cells of the parathyroid glands and secreted as an 84 amino acid moleule called " intact PTH " which is the main bioactive product. This molecule is degraded by proteolytic cleavage between amino acids 33-37 at peripheral site to form biologically active amino terminal fragments which are cleared only by glomerular filtration, while the bioactive intact PTH and amino-terminal fragments are also metabolically degraded in the liver and other tissues. Thus the measurement of intact PTH correlates best with the hormone production and biological activity

Principles and method

The GenWay hPTH-EASIA is a solid phase Enzyme Amplified Sensitivity Immunoassay performed on microtitre plates. Calibrator and samples react with the captured polyclonal antibodies (PAb, goat anti 1-34 PTH fragment) coated on microtitre well. After incubation, the excess of antigen is removed by washing. The monoclonal antibodies (MAb, mouse anti 44-68PTH fragment) labeled with horse radish peroxidase (HRP) are added. After an incubation allowing for the information of a sandwich: coated PAbs–human PTH–Mab – HRP the microtitre plate is washed to remove unbound enzyme labeled antibody. Bound enzyme-labeled anti body is measured through a chromogenic reaction. The chromogenic solution tetramethyl benzydine (TMB) is added and incubated. The reaction is stopped with the addition of stop solution and the microtitre plate is then read at the appropriate wavelength. The amount of the substrate turnover is determined

colorimetrically by measuring the absorbance, which is proportional to the PTH concentration. A calibration curve is plotted and PTH concentration in samples is determined by interpolation from the calibration curve.

2.6 HAEMATOLOGICAL VARIABLES

Various haematological parameters including white blood cell count (WBC), lymphocyte count (LYM), mid cell count (MID), granulocyte count (GRAN), red blood cell count (RBC), haemoglobin concentration (HGB), packed cell volume (PCV), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), red cell distribution width (RDW), and platelet concentration (PLT) were determined by an automated blood analyzer CELL-DYN 1700®, version 1.08, (Abbott Diagnostics, Abbott Park, Illinois, USA).

CELL-DYN hematology autoanalyzer relies primarily on flow cytometry to determine the WBC count and five-part differential count. This technique is based on the fact that the amount of light scattered at different specific angles is characteristic of the different sub-populations of WBCs. A helium neon laser is used as a light source and a series of mirrors, lenses, and slits guide and shape the beam along the light path. When cells pass through the beam of light, the light is scattered. Photo detectors measure the amount of light deflected at specific angles and the data are displayed on scattergrams.

On CELL-DYN analyzers, the MCV is essentially a measured parameter, derived from the average volume of the red blood cells, measured individually. This parameter is an important indicator of the average size of the RBCs in the sample, and thus how much room there is in each cell to transport oxygen. CELL-DYN analyzers also measure the HGB and RBC. The MCH and MCHC are calculated, and represent the average weight of hemoglobin in each red cell (MCH) and the average concentration or percent of haemoglobin in the RBCs (MCHC). The haematocrit is calculated using the MCV and RBC. Measured parameters are determined by a direct analysis or count, and calculated parameters are

determined by a mathematical manipulation of measured parameters or scientific constants (CELL-DYN analyzers manual).

2.7 OXIDATIVE STRESS MARKERS AND ANTIOXIDANTS

Parameters measured included; Malondialdehyde (MDA) (µmol/l), Vitamin C (Vit C) (mg/ml), Catalase (CAT) (units/ml) and Vitamin A (Vit A) (µg/ml).

2.7.1 Malondialdehyde (MDA)

Principle and Method

Malondialdehyde (MDA) levels were determined by the MDA Thiobarbituric acid (TBA) test which is the colorimetric reaction of MDA and TBA in acid solution. MDA, a secondary product of lipid peroxidation, reacts with thiobarbituric acid (TBA) to generate a red coloured product, which was detected spectrophotometrically at 535 nm. This method is a fast, sensitive and low cost method that can be used to indicate the extent of lipid peroxidation in a variety of systems (Shlafer and Shepard, 1984). The protocol used in this study was that of Kamal *et al.*,(1989) as modified by Schlafer and Shepard (1984) protocol which is as follows: Half a millilitre (0.5 ml) of the patient's serum was treated with 2.5 ml of 20% TCA and then 1 ml of 0.67% of thiobarbituric acid (TBA) added. The mixture was incubated at 100°C for 30 minutes. After cooling, the sample was extracted with 4 ml n-Butanol (product number 334790 supplied by BDH Chemicals Limited, Poole, England) and centrifuged at 500 g for 15 minutes. The absorbance of the extracts was measured at 535 nm and the results were expressed as umol/l, using the extinction coefficient of 1.56×10^5 L $mmol^{-1}$ cm^{-1}.

2.7.2 Vitamin C

Vitamin C was determined by the method of Omaye *et al.,* (1979)

Principle and Method

Ascorbic acid in plasma is oxidized by Cu (II) to form dehydroascorbic acid, which reacts with acidic 2, 4 dinitrophenylhydrazine to form a red dihydrazone which is

measured at 520 nm. Ascorbic acid should be analyzed immediately or not later than 3 hours if the specimen is refrigerated. To 0.5 ml of plasma 0.5 ml of water and 1 ml of 5% TCA were added, mixed thoroughly and centrifuged at 500 g for 15 minutes. To 1 ml of the supernatant, 0.2 ml of DTC (0.4 g thiourea, 0.05 g $CuSO_4.5H_2O$, 3 g 2, 4 dinitrophenylhydrazine in 4.5 mol/L H_2SO_4) was added and incubated at 37°C for 3 hours. Then 1.5 ml of 65% sulphuric acid was added mixed and the solution was allowed to stand at room temperature for another 30 minutes. The colour developed was read at 520 nm. The level of vitamin C was expressed as mg/dl of plasma.

2.7.3 Catalase (CAT)

Catalase was assayed by the method of Takahara *et al*.,(1960). To 1.2 ml of 50 mM phosphate buffer (pH 7.0), 0.2 ml of plasma was added and the enzyme reaction was started by the addition of 1.0 ml of 30 mM H_2O_2 solution. The decrease in absorbance was measured at 240 nm at 30 second intervals for 3 minutes. The enzyme blank was run simultaneously with 1.0 ml of distilled water instead of hydrogen peroxide. The enzyme activity was expressed as units/ml.

2.7.4 Vitamin A

Plasma-retinol (vitamin A) was determined by reverse phase high performance liquid chromatography (HPLC) (Zaman *et al*., 1993). This method was used to quantify retinol in a single chromatographic run with an internal standard, Tocol (Lara Spiral, Couternon, France), added for estimation of recovery. The stationery phase was constituted by greffed silica (C18 column, HP ODS Hypersil C18; 200 mm-4.6 mm; Lara Spiral, maintenance temperature of analytical column, 35°C). The mobile phase was a mixture of methanol/water (98/2, v/v) at a flow rate of 1 ml/min. Vitamins were extracted by hexane, dried under nitrogen and resuspended in methanol. The HPLC peaks were detected by an UV detector at 292 nm and 325 nm for vitamin A. Representative chromatograms were obtained by injecting standard solutions. In order to evaluate the daily performance of the

HPLC system, the external standard was injected every day at the beginning, middle and at the end of the chromatographic system.

2.8 RENAL FUNCTION EQUATIONS AND STAGING OF CKD

The seven renal function equations evaluated are listed below; all equations used serum creatinine (SCr) levels to predict renal function.

1. $$Cockroft\ gault = \frac{(140 - age) \times weight}{72 \times SCr} (\times 0.85\ if\ female)$$

2. $$4v - MDRD = 186 \times SCr^{-1.154} \times age^{-0.203} \times (1.212\ if\ black) \times (0.742\ if\ female)$$

3. $$Jelliffe\ 1 = \frac{98 - 0.8 \times (age - 20)}{SCr} (\times 0.90\ if\ female)$$

4. $$Jelliffe\ 2 = Male: 100/Scr - 12$$

$$Female:\ 80/Scr - 7$$

5. $$Bjornson = Male: \frac{[27 - (0.173 \times age)] \times weight \times 0.07}{Scr}$$

$$Female: \frac{[25 - (0.175 \times age)] \times weight \times 0.07}{Scr}$$

6. $$Gates = Male: (89.4 \times Scr^{-1.2}) + (55 - age) \times (0.447 \times Scr^{-1.1})$$

$$Female: (60 \times Scr^{-1.1}) + (56 - age) \times (0.3 \times Scr^{-1.1})$$

7. $$CKD - EPI \quad Female \leq 62 (\leq 0.7) \quad GFR = 166 \times \frac{Scr}{0.7^{-0.329}} (0.993)^{Age}$$

$$> 62 (> 0.7) GFR = 166 \times Scr/0.7^{-1.209} \times (0.993)^{Age}$$

$$Male \leq 80 (\leq 0.9) GFR = 163 \times Scr/0.9^{-0.411} \times (0.993)^{Age}$$

$$> 80 (> 0.9) GFR = 163 \times Scr/0.9^{-1.209} \times (0.993)^{Age}$$

Body surface area (BSA) was estimated according to the method of Du Bois and Du Bois, (1989):

$$BSA = weight(kg)^{0.425} \times height(m)^{0.7250} \times 20247$$

The GFR results from the various renal function equations were used to stratify the study population into five categories corresponding with the five stages of CKD in

the K/DOQI CKD classification (National Kidney Foundation, 2002). The staging classified GFR ≥ 90 ml/min/1.73 m^2 as stage 1; 60-89 ml/min/1.73 m^2 as stage 2; 30-59 ml/min/1.73 m^2 as stage 3; 15-29 ml/min/1.73 m^2 as stage 4; and < 15 ml/min/1.73 m^2 as stage 5.

The 4v MDRD equation was used to estimate the eGFR throughout the study apart from section 3.2 which looked at the predictive performance of the equations.

2.9 CUT-OFFS

Anaemia was defined as haemoglobin ≤ 11.0 g/dl (NKF-K/DOQI, 2006);
Hyperglycaemia, FBG ≥ 6.1 mmol/l;
Hypertriglyceridaemia, TG ≥ 1.7 mmol/l;
Low HDL, HDL-C< 1.0 mmol/l (female), < 0.9 mmol/l (male);
LDL ≥ 160 mmol/l;
Total cholesterol ≥ 5.2 mmol/l

2.9.1 Metabolic Syndrome Definitions

2.9.1.1 National Cholesterol Education Program, Adult Treatment Panel III (NCEP ATP III).

Metabolic syndrome was defined according to the criteria of the National Cholesterol Education Program, Adult Treatment Panel III (NCEP ATP III) to include individuals with three or more of the following five components: (1) abdominal obesity - (waist circumference > 102 cm for men, or > 88 cm for women); (2) high TG ≥ 1.7 mmol/L (150 mg/dl); (3) low HDL-C : men < 0.9 mmol/L (< 40 mg/dl) or women < 1.0 mmol/L (< 50 mg/dl); and (4) High BP (systolic BP ≥ 130 mm Hg or diastolic BP ≥ 85 mm Hg or treatment of hypertension); and (5) high fasting glucose ≥ 6.1 mmol/l (NCEP, 2001a).

2.9.1.2 International Diabetes Federation (IDF)

According to the new definition by the International Diabetes Federation (IDF) (Alberti *et al.*, 2006), metabolic syndrome can be diagnosed if central obesity (waist

measurement: men >90 cm or women >80) is accompanied by any 2 of the following 4 factors:

(1) TG levels of 1.7 mmol/L or greater, (2) an HDL-C cholesterol lower than 1.03 mmol/L for men or lower than 1.29 mmol/L for women, (3) a blood pressure (BP) of 130/85 mm Hg or greater or treatment of previously diagnosed hypertension, and (4) a fasting blood glucose (FBG) of 5.6 mmol/L or greater or previously diagnosed type 2 diabetes.

2.9.1.3 World Health Organization (WHO)

WHO criteria (1999) (Alberti *et al.*, 2006)

(1) Body mass index (BMI) ≥30 kg/m^2 and/or waist-to-hip ratio >0.90 (male), >0.85 (female), (2) blood pressure ≥140/≥90 mmHg or on medication, (3) FBG ≥6.1 mmol/L or on medication for diabetes, impaired glucose tolerance or insulin resistance, (4) triglyceride ≥1.7 mmol/L and/or HDL-C <0.91 mmol/L (male), <1.01 mmol/L (female).

2.10 STATISTICAL ANALYSIS

The results of the various studies were expressed as mean (± SEM). Unpaired t-test was used to compare mean values of continuous variables and $\chi 2$ was used to compare discontinuous variables. Agreement between the predictive equations was assessed by the Bland-Altman statistic (Bland and Altman, 1986). Correlation was assessed by the Pearson's method. Sensitivity and specificity values for the prediction equations in detecting subnormal GFR (i.e. GFR < 60 ml/min/1.73 m^2) were also calculated using Receiver Operator Characteristic (ROC) analysis. To compare differences between stages of CKD, one way analysis of variance (ANOVA) followed by Tukey test to compare all pairs of columns was performed. Relationship between the various electrolytes, electrolyte ratios and parathyroid hormone was assessed by linear regression. Odd ratios (OR's) (with 95% CI) of

CKD by the number of metabolic risk factors was calculated. A level of $p<0.05$ was considered as statistically significant. Multivariate logistic regression was used to calculate odds ratios of risk factors of cardiovascular disease adjusting for age and sex.

GraphPad Prism version 5.00 for windows and SYSTAT version 12 were used for statistical analysis (GraphPad software, San Diego California USA, www.grphpad.com; SYSTAT software, 239 Western Street, Suite F Fairfield, CA, USA, www.*systat*.com).

Chapter 3

RESULTS

3.1 GENERAL DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF STUDY POPULATION

The aetiology of the CKD ranged from diabetic nephropathy, 90 (61.6%) patients; chronic glomerulonephritis, 12 (8.2%) patients; adult polycystic kidney disease, 1 (0.7%) patient; hypertensive nephropathy, 10 (6.8%) patients and chronic kidney disease with unknown aetiology, 33 (22.6%) patients.

The distribution of the demographic and clinical characteristics of the studied population is as shown in Table 3.1. The mean age of the subjects [50.18 ± 1.14 (51.05 ± 1.91 for males and 49.46 ± 1.36 for females)] was not significantly different from that of the control group (46.35 ± 1.96). Apart from serum albumin and eGFR which showed a significantly lower value when the CKD subjects were compared to the control group, proteinuria (PRT), Blood urea nitrogen (BUN), Creatinine (CRT), waist circumference (WC), systolic blood pressure (SBP) and diastolic blood pressure (DBP) showed significantly higher values when CKD subjects were compared to the control group (Table 3.1).

Table 3.1 General demographic and clinical characteristics of study population

Parameter	Control (n=80)	CKD (n=146)
Age (yrs)	46.35 ± 1.96	50.18 ± 1.14
Weight (kg)	65.45 ± 2.43	68.61 ± 2.68
Height (m)	1.63 ± 0.01	1.64 ± 0.01
BMI (kg/m²)	24.66 ± 0.80	24.44 ± 0.44
SBP (mmHg)	120.70 ± 1.82	140.40 ± 3.84***
DBP (mmHg)	70.42 ± 1.25	90.32 ± 2.61***
WC (cm)	74.09 ± 1.71	85.02 ± 1.45*
Proteinuria (mg/l)	0.04 ± 0.02	1.17 ± 0.26***
BUN (mmol/l)	3.51 ± 0.17	15.45 ± 2.80***
Creatinine (µmol/l)	105.90 ± 3.96	268.00 ± 25.60***
eGFR (ml/min/ 1.73 m²)	92.40 ± 5.67	57.61 ± 4.15***
Serum albumin (g/l)	41.50 ± 1.22	36.38 ± 1.21**
BSA (m²)	1.69 ± 0.03	1.73 ± 0.03

Data are given as mean ± SEM, BUN=Blood urea nitrogen, BSA=Body surface area, CKD=Chronic kidney disease, eGFR=estimated glomerular filtration rate, DBP=Diastolic blood pressure, SBP=Systolic blood pressure, WC=Waist circumference when the CKD are compared to the control group. Values significantly different from controls, *P < 0.05; **P < 0.01 and ***P < 0.001

3.2 Predictive Performance of Renal Function Equations among Ghanaians Presenting with Chronic Kidney Disease

Table 3.2 Classification of the study population according to renal function equation.

		e GFR (ml/min/1.73m^2)				
KFE	Total GFR	Stage 1 (≥ 90)	Stage 2 (60-89)	Stage 3 (30-59)	Stage 4 (15-29)	Stage 5 (<15)
4v-MDRD	55.40 ± 4.03(100%)	146.50 ± 9.88(15.7%)	65.68 ± 1.21(24.0%)	42.14 ± 1.28(26.0%)	21.55 ± 1.03(15.1%)	8.78 ± 0.67(18.6%)
CG	48.77 ± 3.10(100%)	125.90 ± 5.71(13.0%)	74.06 ± 2.24(10.9%)	46.35 ± 1.24(39.0%)	22.51 ± 0.74(18.5%)	9.33 ± 0.60(17.8%)
CKD-EPI	51.93 ± 3.32(100%)	125.00 ± 3.74(16.4%)	68.76 ± 1.69(17.8%)	45.45 ± 1.40(32.3%)	20.81 ± 0.85(14.4%)	8.46 ± 0.71(19.2%)
Jelliffe 1	46.97 ± 3.00(100%)	128.40 ± 8.42(10.3%)	71.59 ± 2.06(14.4%)	44.48 ± 1.23(40.4%)	21.58 ± 1.01(16.4%)	9.14 ± 0.62(17.8%)
Jelliffe 2	49.76 ± 3.52(100%)	131.80 ± 6.91(14.4%)	68.17 ± 1.23(18.5%)	44.86 ± 1.36(31.5%)	21.50 ± 1.28(12.3%)	4.73 ± 1.08(22.6%)
Bjornson	50.60 ± 3.64(100%)	133.50 ± 8.70(13.8%)	69.53 ± 1.21(15.2%)	43.50 ± 1.10(37.9%)	21.98 ± 0.91(16.5%)	9.06 ± 0.72(16.5%)
Gates	48.76 ± 3.42(100%)	131.50 ± 7.37(13.1%)	71.85 ± 1.65(14.5%)	44.33 ± 1.13(34.5%)	22.51 ± 0.91(17.2%)	8.25 ± 0.66(20.7%)

Data are presented as Mean ± SEM. RFE = Renal function equation; CG = Cockroft-Gault; 4v-MDRD = 4 variable modification of diet in renal disease; CKD-EPI= Chronic Kidney Disease-Epidemiology collaboration. Figures in parenthesis represent GFR range associated with various stages (1-5) of CKD. Total GFR= mean GFR estimated by individual equations used.

Table 3.2 represents the classification of the study population according to kidney function equation. This analysis was based solely on results of the kidney function equations applied, and all the 146 subjects with CKD were considered. Though there are wide variations in the mean value and percentage subjects with mild CKD (Stage 1 and Stage 2), the CKD-EPI, and CG equations gave similar mean values. The 4v-MDRD equation gave the highest percentage value of 24.0 whilst the Jelliffe 1 equation gave the lowest percentage of 10.3 (Table 3.2). For those with moderate CKD (Stage 3), all the equations gave close mean GFR values apart from the MDRD and Bjornsson equations which turned out lower values. The 4v-MDRD gave the lowest percentage for stage 3(26.0%), whilst the Jelliffe 1 equation gave the highest GFR of 40.4% (Table 3.2). Interestingly, all the renal function equations with the exception of Jelliffe 2 generated similar percentages for subjects with severe CKD (Stage 4 and Stage 5). The 4v-MDRD and CKD-EPI equations however, gave closer comparable percentage values for stage 4 (Table 3.2). Furthermore, the CKD-EPI and MDRD also gave both comparablev mean and percentage values for stage 5.

Table Table 3.3 Pearson's correlation coefficients of clinical variables and kidney function equation for control group (upper right-hand side) and kidney disease group (lower left-hand side).

Parameter	Jalliffe 1	Jalliffe 2	Bjornsson	Gates	CG	CKD-EPI	MDRD	Age	BMI	CRT	ALB	PRT	BUN
Jalliffe 1		0.90***	0.56	0.90	0.34	0.51	0.39	-0.14*	0.16	0.20***	-0.03	-0.03***	0.07
Jalliffe 2	0.89***		0.42	0.95	0.42	0.50	0.40	-0.05*	0.16	0.15***	0.07	0.04*	0.09
Bjornsson	0.55***	-0.11		0.43***	0.13***	0.23***	0.16***	-0.12***	0.04*	0.15	-0.03	0.06	0.04*
Gates	0.90***	0.95***	0.43***		0.46***	0.50***	0.39***	-0.08	0.14*	0.08	0.05	-0.03	0.10*
CG	0.34***	0.42***	0.13	0.46***		0.23***	0.19***	0.05	-0.06*	0.11	-0.10	-0.03	0.09*
CKD-EPI	0.51***	0.50***	0.23**	0.50***	0.79		0.96***	-0.10	0.04*	0.15	-0.08	0.09	0.14*
MDRD	0.39***	0.40***	0.16*	0.40***	0.19*	0.96***		-0.01*	0.02	0.16	-0.05	0.06	0.17*
Age	-0.14	-0.05	-0.12	-0.08	0.05	-0.10	-0.02		0.02	0.00	0.07	-0.07	0.03
BMI	0.16	0.16	0.04	0.14	-0.06	0.04	0.02	0.14		0.05	0.06	0.02	-0.05
CRT	0.20**	0.15	0.15	0.08	0.11	0.15	0.16	0.00	0.05		-0.04*	0.07	0.26*
ALB	-0.03	0.07	-0.03	0.05	-0.10	-0.08	-0.05	0.07	0.06	-0.04		-0.10	0.09
PRT	-0.03	0.04	0.06	-0.03	-0.03	0.09	0.06	-0.07	0.02	0.07	-0.01		0.05
BUN	0.07	0.09	0.04	0.11	0.09	0.14	0.17*	0.03	-0.05	0.26***	0.09	0.05	

CKD-EPI= Chronic kidney disease –Epidemiological initiative; CG=Cockroft-Gault; 4v-MDRD= 4 variable modification of diet in renal disease; BMI= Body mass index; PRT = Proteinuria, BUN =Blood urea nitrogen; CRT = Creatinine; ALB=Albumin. *.Correlation is significant at the 0.05 level (2-tailed), **.Correlation is significant at the 0.01 level (2-tailed), ***.Correlation is significant at the 0.001 level (2-tailed).

From the Pearson correlation analysis (Table 3.3), there are significant positive correlations among the various renal function equations and between BUN and CRT for both the control group and subjects presenting with CKD. However, the relationship between BUN and CRT is stronger within the CKD group than the control. Conversely, the renal function equations generally gave negative but significant correlation with age within only the control group.

Table 3.4 Sensitivity and specificity of equations for GFR< 60ml/min/1.73m²

	eGFR <60ml/min/1.73m²	
KFE	**Sensitivity%**	**Specificity%**
CKD-EPI	66.00	70.00
4v MDRD	67.30	63.90
CG	62.90	80.00
JL1	25.50	74.50
JL2	59.20	69.40
GT	72.41	35.71
BJ	63.00	67.80

CKD-EPI=chronic kidney disease epidemiological initiative; 4v MDRD=4 variable modification of diet in renal disease; CG= Cockroft-Gault; JL1=Jelliffe 1; JL2=Jelliffe 2; GT=Gates; BJ=Bjornsson

Table 3.4 represents sensitivity and specificity of equations for e GFR < 60 ml/min/1.73 m² using reader operator characteristic (ROC) analysis. Considering the various kidney function equations, at least 34.8% of the subjects had GFR < 60 ml/min/1.73 m² (Table 3.2). The sensitivity and specificity of the CKD-EPI equation to detect GFR values less than 60 ml/min/1.73 m² were 66.0% and 70% respectively; that of 4v-MDRD equation to detect GFR values less than 60 ml/min/1.73 m² were 67.3% and 63.9% respectively; that of CG were 62.9% and 80.0% respectively; that of JL1 were 25.5% and 74.5% respectively; that of JL2 were 59.2% and 69.4% respectively; that of GT were 72.41% and 35.71% respectively; that of BJ were 63.0% and 67.8% respectively.

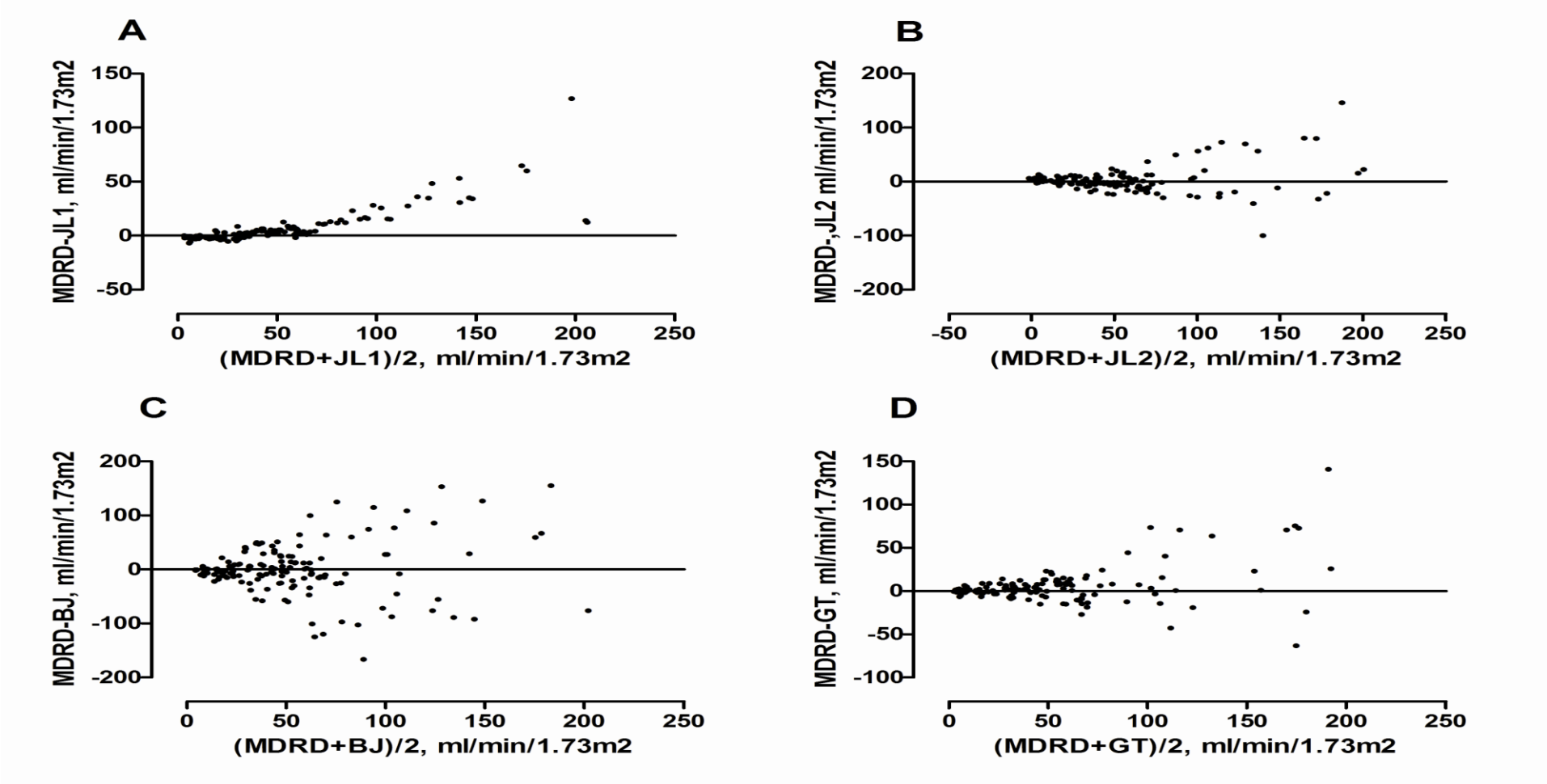

Figure 3.1 Bland-Altman plot showing the agreement between 4v-MDRD and JL 1 (A), 4v-MDRD and JL 2 (B), 4v-MDRD and BJ (C) and 4v-MDRD and Gates (D).

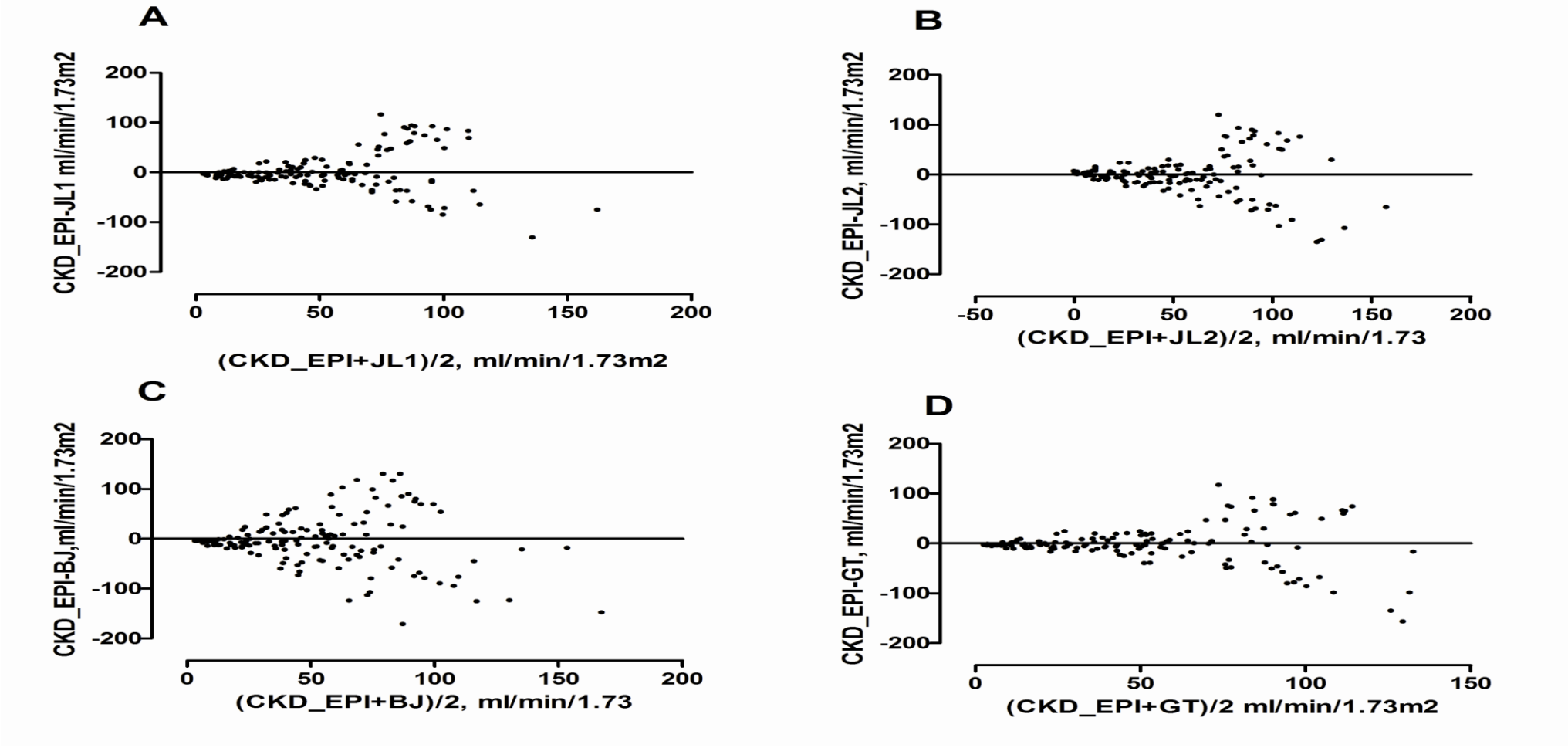

Figure 3.2 Bland-Altman plot showing the agreement between CKD-EPI and JL 1 (A), CKD-EPI and JL 2 (B), CKD-EPI and BJ (C) and CKD-EPI and Gates (D).

Bland-Altman analysis which shows the performance of the various kidney function equations in comparison with 4v-MDRD and CKD-EPI equations are as shown in Figure 3.1 and 3.2 respectively. The bias (i.e. the mean difference) in GFR estimation by the JL 1, JL 2, BJ and Gates equations in relation to CKD-EPI (Figures 3.2 A-D) were 4.6%, 1.3%, -0.7% and 3.3% respectively. Also, the bias in GFR estimation by the JL 1, JL 2, BJ and Gates equations in relation to 4v-MDRD (Figure 3.1 A-D) were 8.0%, 4.8%, 2.7%, and 6.8% respectively. Apart from the agreement between JL 1 and Gates in relation to 4v-MDRD (Figure 3.1 A-D) which were not so good, all the rest gave good agreement in accordance with the results from the Pearson correlation described above in Table 3.3.

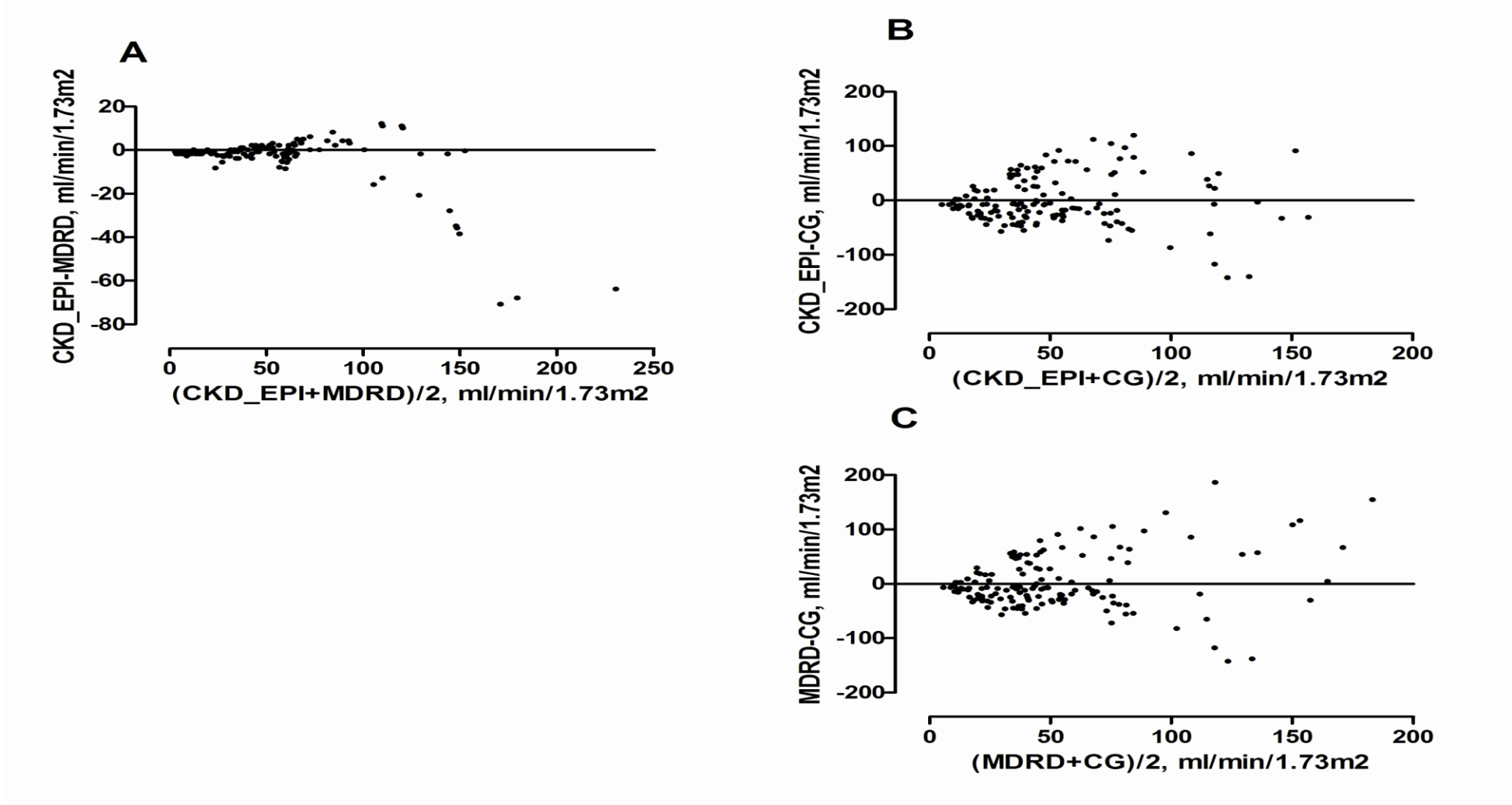

Figure 3.3 Bland-Altman plot showing the agreement between CKD-EPI and 4v MDRD (A), CKD-EPI and CG (B), 4v-MDRD and CG (C).

There was also a good agreement between CKD-EPI and 4v-MDRD equation (bias of -3.1), and 4v MDRD and CG (bias of 4.3) as well as CKD-EPI and CG (bias of 2.2) as shown in Figure 3.3 A and B.

3.3 Metabolic Syndrome among Ghanaian Patients Presenting with Chronic Kidney Disease.

Table 3.5 General characteristics of study population with and without metabolic syndrome

			Metabolic Syndrome		Gender	
Parameters	Control (n=80)	CKD (n=146)	CKD+MetS (n=44)	CKD-MetS (n=102)	CKD Female (n=80)	CKD Male (n=66)
Age (yrs)	46.35 ± 1.96	50.18 ± 1.14	61.00 ± 2.61	44.00 ± 1.65††	49.46 ± 1.36	51.05 ± 1.91
BMI (kg/m^2)	24.66 ± 0.80	24.44 ± 0.44	27.64 ± 1.27	24.77 ± 0.56†	26.24 ± 0.90	24.27 ± 0.59
WC (cm)	74.09 ± 1.71	85.02 ± 1.45*	89.45 ± 3.10	82.34 ± 1.62†	84.64 ± 2.16	84.04 ± 1.90
SBP (mmHg)	120.70 ± 1.82	140.40 ± 3.84***	143.50 ± 4.26	135.6 ± 2.40†	144.7 ± 3.49	136.50 ± 2.84
DBP (mmHg)	70.42 ± 1.25	90.32 ± 2.61***	89.00 ± 2.71	87.26 ± 1.66††	93.36 ± 2.53	87.69 ± 1.77
PRT (g/l)	0.04 ± 0.02	1.17 ± 0.26***	0.71 ± 0.24	1.12 ± 0.17	1.19 ± 0.39	1.15 ± 0.35
CRT (μmol/l)	105.90 ± 3.96	268.00 ± 25.60***	371.2 ± 82.58	353.9 ± 47.48	221.80 ± 25.01	325.30 ± 47.39
FBG (mmol/l)	5.31 ± 0.17	8.75 ± 0.33***	7.80 ± 0.48	6.90 ± 0.27	6.85 ± 0.47	7.24 ± 0.63
HDL-C (mmol/l)	1.35 ± 0.05	1.61 ± 0.20	1.03 ± 0.06	1.46 ± 0.06††	1.43 ± 0.14	1.32 ± 0.12
TG (mmol/l)	1.52 ± 0.08	1.84 ± 0.09*	2.70 ± 0.15	1.88 ± 0.13†	1.84 ± 0.25	2.24 ± 0.27
TC (mmol/l)	4.54 ± 0.13	5.32 ± 0.30*	5.61 ± 0.25	5.35 ± 0.20	5.38 ± 0.38	5.26 ± 0.44
eGFR (ml/min/ 1.73 m^2)	92.40 ± 5.67	57.61 ± 4.15***	99.72 ± 13.43	89.28 ± 6.90	50.16 ± 4.12	66.79 ± 7.63§
Prevalence of MetS	3(3.75%)	44(30.1%)			29(36.2%)	15 (22.7%)

BMI = Body mass index, WC= Waist circumference, SBP = Systolic blood pressure, DBP = Diastolic blood pressure, PRT = Proteinuria, CRT = Creatinine, TC = Cholesterol, HDL-C = High density lipoprotein, TG = Triglyceride, FBG = Fasting blood glucose, eGFR = Glomerular filtration rate, MetS = Metabolic syndrome. CKD+MetS=CKD patients with MetS; CKD-MetS=CKD patients without MetS.*p<0.05, **p<0.01, ***p<0.001; †p<0.05, ††p<0.01; §p<0.05 when the groups were compared.

From this study, the CKD subjects had significantly higher levels of urine protein, serum creatinine and lower levels of estimated GFR as compared to the control subjects (Table 3.5); however there was no significant difference between the CKD subjects and controls in relation to age, BMI and HDL-C. The mean values of most indices of the metabolic syndrome were significantly higher when the CKD group were compared to the control group i.e. the CKD group had significantly higher WC, had higher blood pressure [systolic blood pressure (SBP) and diastolic blood pressure (DBP)], higher fasting blood glucose (FBG) and had higher lipid levels (i.e. TG and TC) than the control group (Table 3.5). 30.1% of the CKD subjects had metabolic syndrome compared to 3.75% of the controls (Table 3.5).

When CKD patients were stratified according to the presence or absence of the MS, those with the metabolic syndrome were significantly older and had higher BMI, SBP, WC, DBP and TG as compared to those without MetS as shown in Table 3.4. The mean value of HDL-C was significantly lower among those with MetS as compared to those without MetS (Table 3.5).

However, when the CKD patients were classified by gender, the female subjects had significantly lower estimated GFR as compared to the male subjects. The percentage of female CKD patients with the MetS is 36.2% compared to 22.7% among the males (Table 3.5).

Table 3.6 Clinical and metabolic characteristics of CKD patients according to different definitions of the metabolic syndrome

	Type of definition of MetS		
Parameter	IDF	WHO	NCEP ATP III
Mean WC/BMI	85.06 ± 1.46	24.47 ± 0.44	88.58 ± 3.31
Raised WC/BMI	93.88 ± 1.45	33.27 ± 0.55	103.70 ± 1.82
TG	2.65 ± 0.12	2.66 ± 0.12	2.66 ± 0.12
HDL-C	0.95 ± 0.03	0.95 ± 0.03	0.69 ± 0.03***
FBG	9.75 ± 0.36	10.52 ± 0.38	10.52 ± 0.38
Mean SBP	156.7 ± 2.67	148 ± 2.3	156.7 ± 2.67
Mean DBP	97.50 ± 1.64	97.50 ± 1.64	97.50 ± 1.64
Raised BP	30.80%	26.70%	30.80%
MetS	45.20%	19.20%	30.10%

**=p<0.05; **p< 0.01; ***p<0.001, IDF=International Federation of Diabetes; WHO=World Health Organization; NCEP ATP III=National Cholesterol Education Program Adult Treatment Panel III; MetS=Metabolic syndrome.*

Table 3.6 represents the clinical and metabolic characteristics of CKD patients according to different definations of the metabolic syndrome. Mean low HDL-C was significantly lower by NCEP ATP III definition compared to IDF and WHO definition. The IDF, WHO, NCEP ATP II definitions classified 45.20%, 19.20% and 30.10% respectively of the CKD population as having the MetS.

Table 3.7 Odds Ratios of MetS risk factors in CKD stratified by presence/absence of MetS or gender.

Groups/OR	Raised Blood Pressure	Raised Fasting Glucose	Obesity	Raised Triglyceride	Reduced HDL-C-	Proteinuria
Control (n=80)	4/80(5.5%)	14/80(17.5%)	13/80(16.2%)	22/80(27.5%)	4/80(5.0%)	0/80(0.0%)
CKD (n=146)	45/146(30.8%)	97/146(66.4%)	51/146(35.0%)	69/146(47.2%)	28/146(19.2%)	105/146(72%)
OR(95% CI)	8.9(3.1- 25.1)***	9.3(4.7-18.2)***	2.7(1.4-5.5)**	2.3(1.3-4.2)**	4.5(1.5-13.4)**	409(24.7-6759)***
			Stratified based on metabolic syndrome			
CKD-MetS (n=102)	19/102(18.6%)	62/102(60.7%)	29/102(28.4%)	30/102(29.4%)	37/102(36.2%)	30/102(29.4%)
CKD+MetS (n=44)	25/44(56.8%)	34/44(77.2%)	20/44(45.4%)	33/44(75.0%)	34/44(77.3%)	17/44(38.6%)
OR(95% CI)	5.7(2.6-12.5)***	2.2(0.9-4.9)	2.1(1.0-4.3)	7.2(3.2-16.1)***	6.0(2.6-13.4)***	1.5(0.7-3.1%)
			Stratified by gender			
CKD+ Female (n=80)	25/80(31.2%)	52/80(65.0%)	28/80(35.0%)	45/80(56.2%)	16/80(20.0%)	64/80(80.0%)
CKD+ Male (n=66)	20/66(30.3%)	45/66(68.2%)	8/66(12.1%)	34/66(51.5%)	16/66(24.2%)	40/66(60.6%)
OR(95% CI)	0.9(0.5-1.9)	1.1(0.6-2.3)ns	0.2(0.1-0.6)**	0.8(0.4-1.6)	1.6(0.7-3.5)	0.4(0.2-0.8)*

HDL-C = High density lipoprotein cholesterol, CKD = Chronic kidney disease, OR = Odds ratio, CI = Confidence interval. CKD+MetS=CKD patients with metabolic syndrome; CKD-MetS=CKD patients without metabolic syndrome.*p<0.05, **p<0.01, ***p<0.001.

3.3.1 Relative risk of developing metabolic syndrome risk factors

Table 3.7 represents the odds ratios of MetS risk factors in CKD stratified by the presenceabsence of MetS and gender. As compared to the control subjects, the CKD patients were about 9 fold at risk of developing hypertension (OR = 8.9; 95% CI = 3.1-25.1) and diabetes (OR = 9.3; 95% CI = 4.7-18.2), about 2 times (OR = 2.3; 95% CI = 1.3-4.2), 3 times (OR = 2.7; CI = 1.4-5.5) and approximately 4 times (OR=4.5;95% CI = 1.5-13.4) at risk of having hypertriglyceridaemia, obesity and low HDL respectively (Table 3.7). The risk of developing proteinuria is 400 folds in the CKD patients compared to the controls (OR = 409; CI = 24.7-6759).

When the CKD patients were stratified based on the presence or absence of the metabolic syndrome, those with MetS were about 6 times at risk of having hypertension (OR = 5.7; 95% CI = 2.9-16.8) and reduced HDL-C (OR = 6.0; CI = 2.6-13.4); and 7 times at risk of having raised triglycerides (OR = 7.2; CI = 3.2-16.1) (Table 3.6). The risk of developing obesity (OR = 0.2; 95% CI = 0.1-0.6) and proteinuria (OR = 0.4; 95% CI = 0.2-0.8) is more pronounced in the CKD females compared to the males.

Table 3.8 Odds ratios of MetS risk factors at various stages of CKD.

Parameter	Stage 1 (n=24)	OR	Stage 2 (n=35)	OR	Stage 3 (n=37)	OR	Stage 4 (n=25)	OR	Stage (n=24)	OR
Hypertension	8/24(33.3%)	9.5(2.5-35.4)	9/35(25.7%)	6.6(1.8-23.2)	12/37(32.4%)	9.1(2.7-30.8)	6/25(24.0%)	6.0(1.5-23.4)	10/24(41.6%)	13.6(3.7-49.4)
FBG	13/24(54.1%)	5.5(2.1-15.0)	26/35(74.3%)	13.6(5.2-35.3)	28/37(75.6%)	14.6(5.7-37.8)	18/25(72.0%)	12.1(4.2-34.5)	12/24(50.0%)	4.7(1.7-12.6)
Obesity	5/24(20.8%)	1.3(0.4-4.3)	8/35(22.8%)	1.5(0.5-4.1)	9/37(24.3%)	1.6(0.6-4.3)	10/25(40.0%)	3.4(1.2-9.3)	4/24(16.7%)	1.0(0.3-3.5)
TG	10/24(41.6%)	1.8(0.7-4.8)	18/35(51.4%)	2.8(1.2-6.4)	18/37(48.6%)	2.5(1.1-5.6)	10/25(40.0%)	1.7(0.7-4.5)	13/24(54.1%)	3.1(1.2-8.0)
Low HDL-C	4/24(16.7%)	3.8(0.8-16.5)	5/35(14.3%)	1.9(0.8-12.6)	11/37(29.7%)	8.0(2.3-27.4)	5/25(20.0%)	4.7(1.2-19.3)	7/24(29.1%)	7.8(2.0-29.8)
Proteinuria	5/24(20.8%)	45.0(2.4-857)	12/25(48.0%)	149(8.3-2671)	12/37(32.4%)	79(4.5-1381)	10/25(40%)	109(6.0-1961)	7/24(29.1%)	69(3.7-1266)
MetS	6/24(25.0%)	8.5(1.9-37.5)	13/35(37.1%)	15.1(3.9-58.0)	13/37(35.1%)	14.0(3.6-52.9)	4/25(16.0%)	4.8(1.0-23.5)	8/24(33.3%)	12.8(3.0-53.7)

TG = Triglycerides; HDL-C = High density lipoprotein cholesterol; MetS = Metabolic syndrome; OR = Odds ratio; eGFR =estimated GFR; Stage 1 = eGFR ≥90 mL/min/1.73m^2; stage 2 = eGFR 60-89 mL/min/1.73m^2; stage 3 = eGFR 30-59 mL/min/1.73m^2; stage 4 = eGFR 16-29 mL/min/1.73m^2; stage 5 = eGFR<15 mL/min/1.73m^2 or dialysis.

Table 3.8 represents the odds ratios of MetS risk factors at various stages of CKD. When the CKD subjects were classified into the various stages, the risk of developing hypertension decreased from about 10 times in stage 1 to about 7 times in stage 2 before increasing to about 9 times for stage 3, decreased to 6 times in stage 4 and increased to about 14 times in stage 5. The risk of having hyperglycaemia also increased from stage 1 to stage 3, and then decreased in stage 4 and 5, whereas the risk of developing obesity remained fairly stable throughout the various stages (1-5). The risk of developing low HDL-C decreased from stage 1 to stage 2 before increasing in stage 3, with a further decrease in stage 4, and finally increasing again at stage 5. The risks of developing hypertriglyceridaemia slightly increased progressively reaching the highest value at stage 5. MetS risk increased and reached a peak at stage 3, and decreased at stage 4 before finally increasing again at stage 5. The risk of developing proteinuria from this study fluctuated through the stages reaching a value greater than the initial value at stage 5.

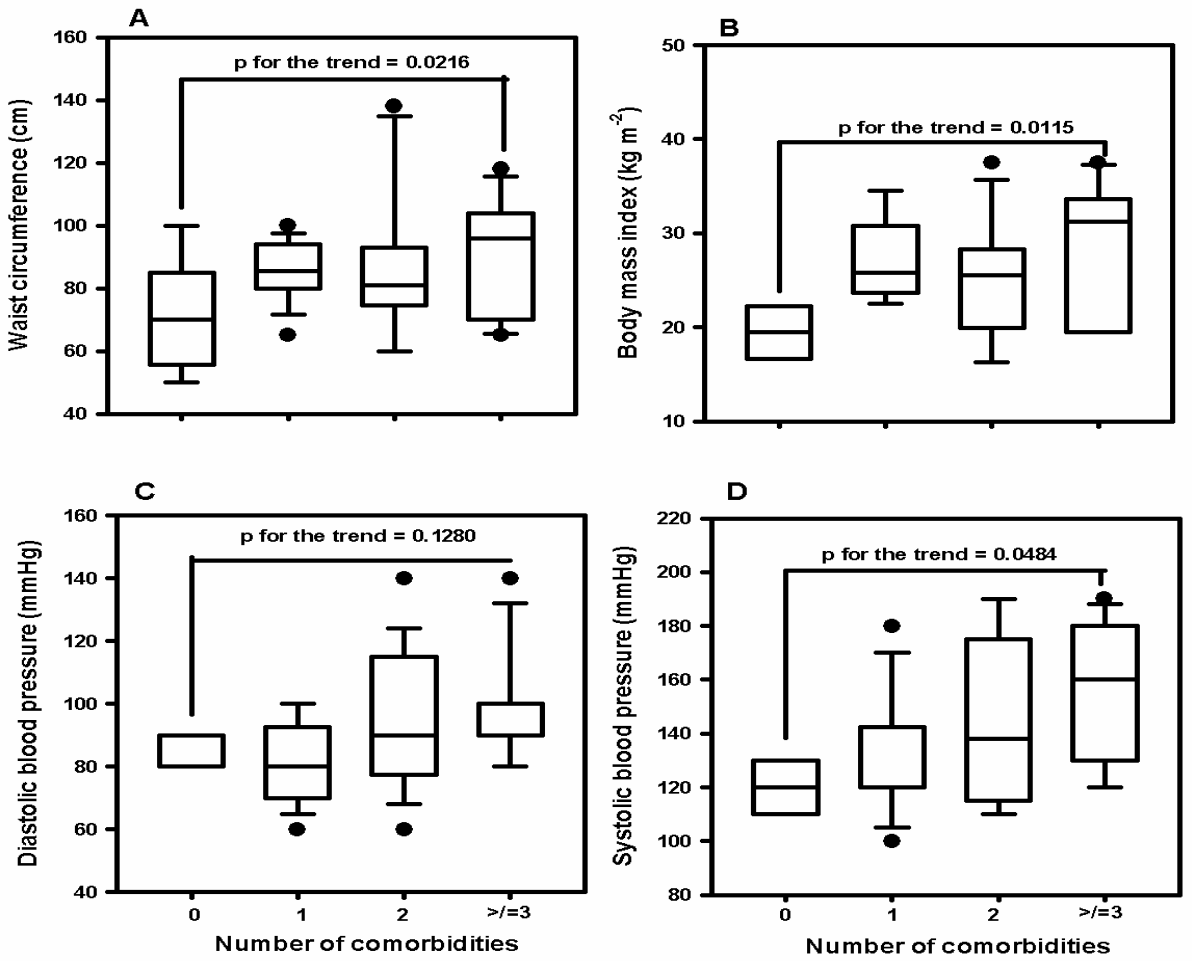

Figure 3.4 *Comparisons of body mass index (BMI) (A), diastolic blood pressure (SBP) (C), systolic blood pressure (SBP) (D) and waist circumference (WC) (B) between patients with a different number of comorbidities of the MetS in CKD. The lower and upper margins of the box represent the 25th and 75th percentiles, with the extended arms representing the 10th and 90th percentiles, respectively. The median is shown as the horizontal line within the box. Outlying points are shown individually.*

Many of the patients had multiple comorbidities, and patients with a greater number of comorbidities also had higher WC ($F_{3,46}$ = 2.878; p =0.0216), BMI ($F_{3,46}$ = 4.112;p= 0.0115) and SBP levels ($F_{3,43}$ =2.546; p=0.0484) as shown in Figure 3.4. For those having zero, one, two or at least three comorbidities, the WC levels were 68.13±4.74, 86.43±2.48, 86.65±5.35 or 89.45±5.54, respectively. The BMI levels were 19.17±1.03, 27.31±1.19, 25.35±1.56 or 27.64±2.28 for those with zero, one, two or at least three comorbidities, respectively. The SBP levels for those with zero, one, two or at least three comorbidities were 124.00±4.00, 131.40±5.73, 143.40±7.28 or 154.5±7.67, respectively. However, DBP showed no significant difference (p=0.1280).

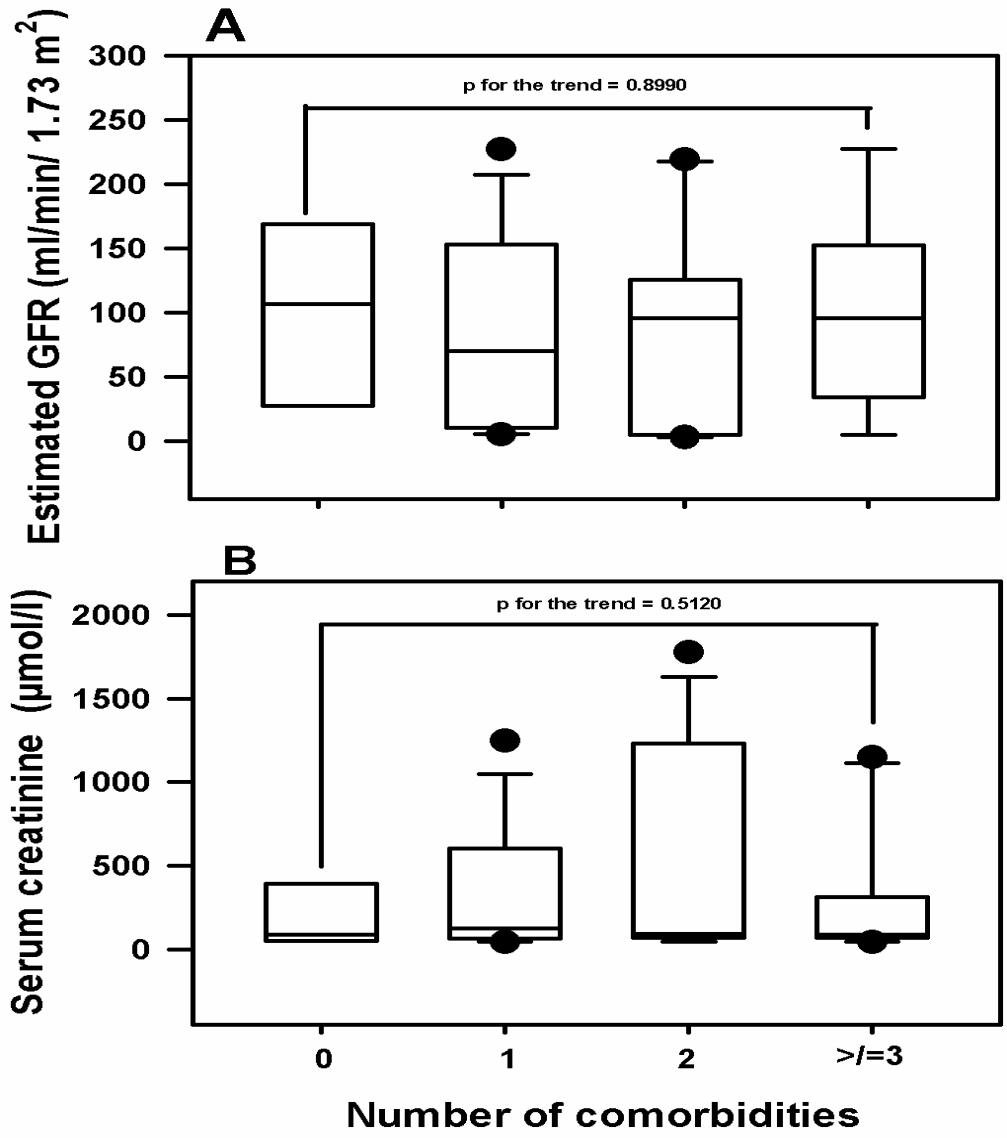

Figure 3.5 Comparisons of estimated GFR (3A) and serum creatinine levels (B) between patients with a different number of comorbidities of the MetS in CKD. The lower and upper margins of the box represent the 25th and 75th percentiles, with the extended arms representing the 10th and 90th percentiles, respectively. The median is shown as the horizontal line within the box. Outlying points are shown individually.

From Figure 3.5, serum CRT ($F_{3,44}$ = 0.7791; p = 0.5120) and eGFR ($F_{3,42}$ = 0.1953; p = 0.8990) showed no significant difference for trend. For those having zero, one, two or at least three comorbidities, the eGFR levels were 108.30±28.38, 87.55±20.60, 86.38±17.72 and 99.72±24.21, respectively. The serum CRT levels were 216.60±81.14, 311.60±103.70, 485.80±159.90 or 263.30±122.30 for those with zero, one, two or at least three comorbidities respectively.

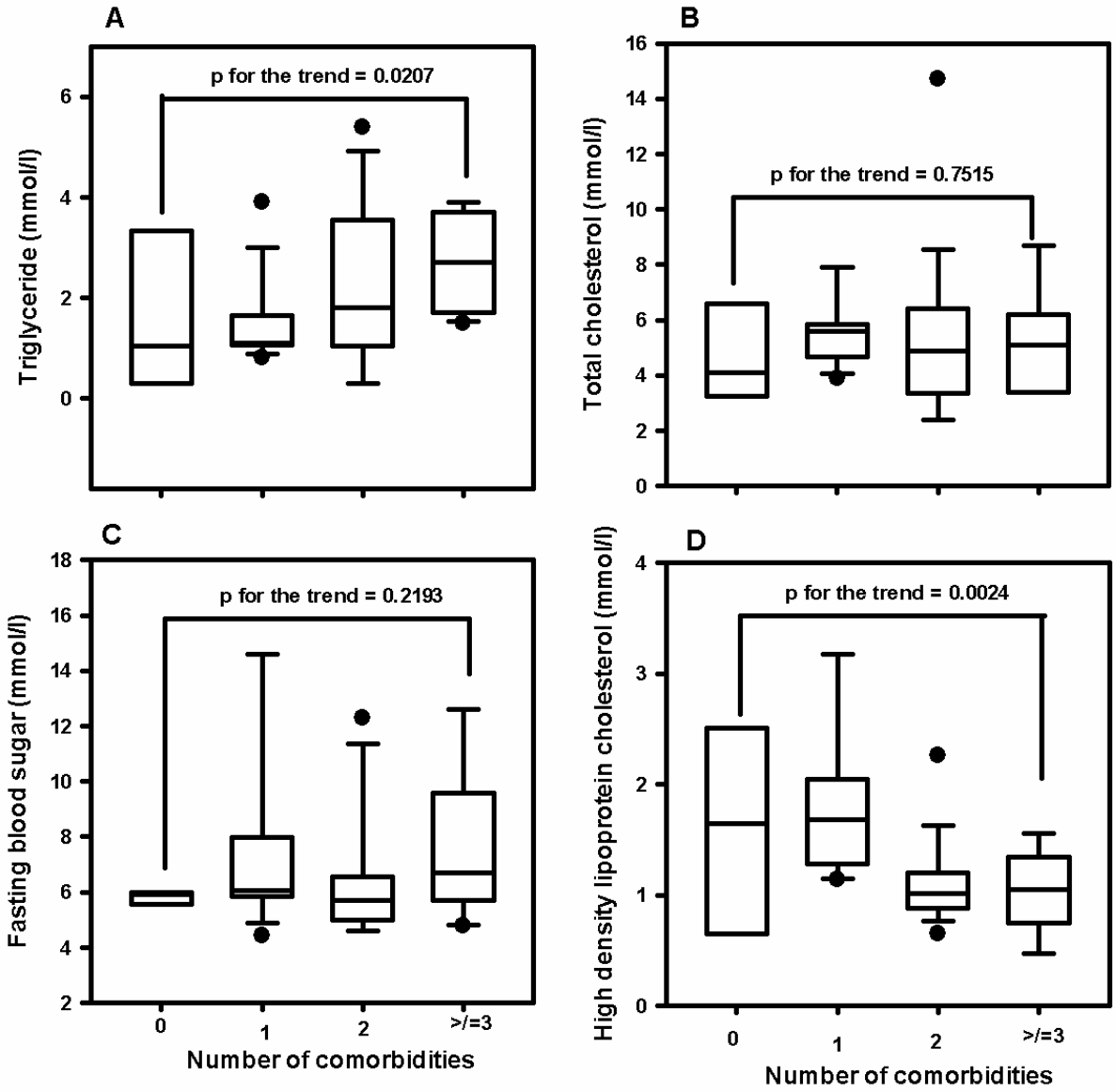

Figure 3.6 Comparisons of fasting blood glucose (C), triglycerides (A), total cholesterol (B) and high density lipoprotein (D) cholesterol levels between patients with a different number of comorbidities of the MetS in CKD. The lower and upper margins of the box represent the 25th and 75th percentiles, with the extended arms representing the 10th and 90th percentiles, respectively. The median is shown as the horizontal line within the box. Outlying points are shown individually.

Many of the patients had multiple comorbidities, and patients with a greater number of comorbidities also had higher TG ($F_{3,45}$ = 3.593; p = 0.0207) and lower HDL-C ($F_{3,46}$ = 5.573; p = 0.0024) (Figure 3.6). However, FBG ($F_{3,44}$ = 1.533; p = 0.2193) and TC ($F_{3,46}$ = 0.4027; p = 0.7517) showed no significant difference for trend. The TG levels were 1.23±0.50, 1.44±0.21, 2.41±0.40 or 2.70±0.27 for those with zero, one, two, or at least three comorbidities, respectively. The low HDL-C levels for those with zero, one, two or at least three comorbidities were 1.58±0.31, 1.81±0.18, 1.11±0.08 or 1.03±0.11 respectively.

3.4 Anaemia as a Risk Factor for Cardiovascular Disease in Patients with Chronic Kidney Disease

Table 3.9 Demographic and clinical characteristics of study population

Parameters	Control (n=80)	CKD (n=146)	P value
Age (yrs)	46.35 ± 1.96	50.18 ± 1.14	0.0720
BMI (kg/m^2)	24.66 ± 0.80	24.44 ± 0.44	0.5270
SBP (mmHg)	120.70 ± 1.82	140.40 ± 3.85	<0.0001
DBP (mmHg)	70.21 ± 1.21	90.32 ± 2.61	<0.0001
PRT (g/dl)	0.04 ± 0.02	1.17 ± 0.26	<0.0001
HGB (g/dl)	12.45 ± 0.19	10.51 ± 0.20	<0.0001
HCT (%)	35.04 ± 0.42	33.18 ± 0.60	0.0023
RBC (k/µl)	4.60 ± 0.07	3.64 ± 0.16	<0.0001
TC (mmol/l)	4.53 ± 0.13	5.63 ± 0.13	<0.0001
TG (mmol/l)	1.56 ± 0.07	1.84 ± 0.09	0.0036
HDL-C (mmol/l)	1.35 ± 0.05	1.61 ± 0.20	0.2114
LDL-C (mmol/l)	2.75 ± 0.10	3.30 ± 0.14	0.0134
AI	2.40 ± 0.12	3.21 ± 0.23	0.0019
FBG (mmol/l)	5.31 ± 0.17	8.75 ± 0.33	<0.0001
CRT (µmol/l)	105.90 ± 3.96	268.20 ± 25.60	<0.0001
eGFR (ml/min per 1.73 m^2)	92.40 ± 5.67	57.61 ± 4.15	<0.0001

SBP=Systolic blood pressure, DBP=Diastolic blood pressure, FBG=Fasting blood glucose, HCT= Haematocrit, HGB=Haemoglobin, RBC=Red blood cell, CRT=Creatinine, PRT=Proteinuria, BMI=Body mass index, TC= Total cholesterol, TG=Triglyceride, AI=Atherogenic index, HDL-C=High density lipoprotein cholesterol, LDL-C= Low density lipoprotein cholesterol, eGFR=Estimated glomerular filtration rate.

Table 3.10 Demographic and biochemical characteristics of study population stratified by the presence or absence of CKD.

		CKD stratification		
Parameters	**Total (n=146)**	**GFR>60 (n=60)**	**GFR<60 (n=86)**	**P value**
Demographics				
Age (yrs)	50.18 ± 1.14	49.60 ± 2.05	50.69 ± 1.34	0.6412
Male (%)	45.20%	40.9%	57.5%	0.6035
Medical history and examination				
Hyperglycaemia (%)	66.40%	65.00%	87.20%	0.0021
Hypertension (%)	30.8%	28.30%	32.5%	0.7159
Mean SBP (mmHg)	140.40 ± 3.85	137.00 ± 6.98	141.80 ± 4.66	0.5725
SBP ≥ 140 mmHg (%)	52.00%	48.60%	40.00%	0.7582
Mean DBP (mmHg)	90.32 ± 2.61	90.45 ± 3.42	90.00 ± 3.63	0.9375
DBP ≥ 90 mmHg (%)	58.00%	51.40%	53.30%	1.0000
Mean BMI (kg/m^2)	24.44 ± 0.44	24.69 ± 0.60	24.24 ± 0.62	0.6443
Laboratory values				
Mean CRT (µmol/l)	268.20 ± 25.60	90.73 ± 3.62	389.90 ± 37.91	<0.0001
Mean HGB (g/dl)	10.51 ± 0.2	12.45 ± 0.50	11.01 ± 0.23	<0.0001
Anaemia (%)	53.40%	48.30%	55.80%	0.0138
Mean HCT (%)	30.09 ± 1.43	31.10 ± 1.63	27.74 ± 2.86	<0.0001
Mean RBC (k/µl)	3.64 ± 0.16	4.09 ± 0.17	3.00 ± 0.34	<0.0001
Mean LDL-C (mmol/l)	3.26 ± 0.14	3.70 ± 0.35	2.70 ± 0.31	0.0899
LDL-C > 4.14 (%)	22.00%	22.3%	21.60%	0.0787
Mean HDL-C(mmol/l)	1.61 ± 0.20	1.37 ± 0.11	1.37 ± 0.18	0.9981
HDL-C < 1.3 (%)	51.3%	43.30%	57.00%	0.1303
Mean TC (mmol/l)	5.63 ± 0.13	5.62 ± 0.12	5.53 ± 0.14	0.7132
TC ≥ 5.2 (%)	56.20%	36.67%	58.14%	0.0121

BMI = Body mass index; SBP=Systolic blood pressure; DBP= Diastolic blood pressure; PRT= Proteinuria; HGB=Haemoglobin; HCT=Haematocrit; TC= Cholesterol; HDL-C=High density lipoprotein; TG=Triglyceride; LDL-C=Low density lipoprotein; AI=Atherogenic index; CRT= Creatinine; GFR=Glomerular filtration rate; FBG=Fasting blood sugar; RBC=Red blood cell.

The demographic and clinical characteristics of study population are as shown in Table 3.9. The mean age of the 146 participants with CKD included in our study was 50.18±1.14 years, with 45.2% of participants being males. Apart from the haematological parameters (Haemoglobin (HGB), Haematocrit (HCT), and RBC) and GFR which were significantly decreased as compared to the control, lipid fractions (TC, TG and LDL-C), FBG and blood pressure were significantly increased when the CKD subjects were compared to the control group.

When the study population was stratified by the presence/absence of CKD (Table 3.10) approximately 58.9% of the subjects had an estimated GFR (eGFR) of < 60 ml/min/1.73 m^2. Compared to subjects with an eGFR ≥ 60 ml/min/1.73m^2, those with an eGFR < 60 ml/min/1.73 m^2 were more likely to be anaemic and hyperglycaemic. Subjects with eGFR < 60 ml/min/1.73m^2 also had higher mean values for serum creatinine and lower mean values for haematological parameters (HGB, HCT and RBC). The mean values of LDL-C cholesterol, HDL-C cholesterol and total cholesterol did not appear to vary substantially with estimated GFR.

Table 3.11 Demographic and biochemical characteristics of study population stratified by the presence or absence of anaemia

	Study Population		
Parameters	**CKD-Anaemia (n=68)**	**CKD+Anaemia (n=78)**	**P value**
Demographics			
Age (yrs)	43.04 ± 3.53	52.00 ± 1.30	<0.0001
Male (%)	48.70%	58.50%	0.0456
Medical history/examination			
Hyperglycaemia (%)	50.00%	75.00%	0.0034
Hypertension (%)	19.10%	41.02%	0.0067
Mean SBP (mmHg)	134.01 ± 2.98	143.50 ± 2.44	0.0274
SBP ≥ 140 mmHg (%)	23.50%	44.30%	0.0095
Mean DBP (mmHg)	79.53 ± 1.66	90.17 ± 1.76	0.0764
DBP ≥ 90 mmHg (%)	77.20%	79.40%	0.842
Mean BMI (kg/m^2)	25.53 ± 0.74	23.86 ± 0.54	0.1338
Laboratory values			
Mean CRT (μmol/l)	245.00 ± 32.36	286.50 ± 38.70	<0.0001
Mean HGB (g/dl)	12.33 ± 0.21	8.98 ± 0.50	<0.0001
Mean HCT (%)	38.01 ± 0.66	21.97 ± 1.73	<0.0001
Mean RBC (k/μl)	4.39 ± 0.08	3.01 ± 0.22	<0.0001
Mean LDL-C (mmol/l)	2.91 ± 0.17	3.70 ± 0.23	0.0062
LDL-C > 4.12 (%)	14.10%	30.80%	0.013
Mean HDL-C (mmol/l)	1.72 ± 0.30	1.52 ± 0.26	0.0999
HDL -C< 1.3 (%)	48.52%	53.80%	0.6188
Mean TC (mmol/l)	4.81 ± 0.32	5.65 ± 0.13	0.0015
TC ≥ 5.2 (%)	29.40%	60.25%	0.0002
eGFR (ml/min/1.73m^2)	70.46 ± 6.67	56.86 ± 5.46	0.0042

SBP=systolic blood pressure; DBP=diastolic blood pressure; BMI=body mass index; CRT=creatinine; HGB=haemoglobin; HCT=haematocrit; RBC=red blood cell; LDL-C=low density lipoprotein cholesterol; HDL-C=high density lipoprotein cholesterol; TC=total cholesterol; eGFR=estimated glomerular filtration rate; Anaemia-CKD=CKD patients without anaemia; Anaemia+CKD=CKD patients with anaemia.

The demographic and biochemical characteristics of the CKD cohort stratified by the presence or absence of anaemia are shown in Table 3.11. Subjects with anaemia had a higher prevalence of several CVD risk factors, including older age, hyperglycaemia and hypertension. Subjects with anaemia as expected also had significantly lower values for haematological parameters (HGB, HCT and RBC) and eGFR. Also, they had higher CRT, TC and LDL-C levels compared with subjects without anaemia. The mean value of HDL-C did not appear to vary substantially in the absence/presence of anaemia.

Table 3.12 Cardiovascular risk factors stratified by presence/absence of anaemia and CKD

Parameters	Subjects			
	- Anaemia - CKD (n=30)	**-Anaemia +CKD (n=38)**	**+ Anaemia -CKD (n=30)**	**+Anaemia +CKD (n=48)**
SBP	11(47.8%)	13(34.2%)	16(53.3%)	34(70.8%)
DBP	6(26.1%)	10(26.3%)	12(40.0%)	22(45.8%)
Obesity-BMI	6(20.0%)	6(21.0%)	3(17.6%)	8(16.7%)
Hypercholesterolaemia	15(65.2%)	21(55.2%)	13(43.3%)	30(62.5%)
Hypertriglyceridaemia	17(56.6%)	19(50.0%)	11(36.7%)	21(43.7%)
Low HDL-C	11(36.6%)	22(57.9%)	15(50.0%)	27(56.2%)
Hyperglycaemia	18(47.8%)	29(76.0%)	16(53.3%)	26(54.1%)
Renal Insufficiency	10(33.3%)	38(100%)	1(6.6%)	48(100%)

BMI=Body mass index; SBP=Systolic blood pressure; DBP= Diastolic blood pressure; HDL=High density lipoprotein; +Anaemia=Presence of anaemia; -Anaemia=absence of CKD; +CKD=Presence of CKD; -CKD=Absence of CKD.

Table 3.12 represents cardiovascular risk factors stratified by presence/absence of CKD. Using participants without anaemia and CKD as the reference group, participants with only CKD were particularly at risk of developing hypertriglyceridaemia, hypercholesterolaemia, low HDL-C, diabetes, renal insufficiency and less likely to develop obesity. Those with only anaemia were at risk of developing hypercholesterolaemia, low HDL-C, diabetes and less likely to develop obesity and renal insufficiency. Subjects with both anaemia and CKD were at particularly high risk for developing hypertension, low HDL-C, diabetes, hypercholesterolaemia and renal insufficiency.

Table 3.13 Pearson correlation coefficients of clinical variables and demographic characteristics for chronic kidney disease (upper right-hand side) and control group (lower left-hand side).

	Pearson correlation coefficients													
Parameter	**BMI**	**SBP**	**DBP**	**PRT**	**HGB**	**HCT**	**TC**	**TG**	**HDL**	**LDL**	**CRT**	**eGFR**	**FBG**	**RBC**
BMI		0.05	0.03	0.02	0.17	0.10	-0.21	-0.13	-0.11	-0.18	-0.06	-0.01	0.08	-0.13
SBP	0.39**		0.76***	0.10	0.10	0.12	-0.06	0.02	-0.09	-0.10	-0.02	0.06	-0.18	0.13
DBP	0.43**	0.63***		0.03	-0.02	-0.01	-0.16	0.03	-0.25	-0.16	0.11	0.19	-0.12	0.01
PRT	-0.05	0.23	0.05		-0.03	-0.03	-0.06	-0.16	0.01	-0.03	-0.17	0.06	-0.06	0.01
HGB	-0.08	0.25	0.08	-0.018		0.99***	-0.16	0.12	-0.10	-0.21	-0.30*	0.13	-0.06	0.92***
HCT	-0.03	0.25	0.08	-0.16	0.96***		-0.15	0.13	-0.13	-0.20	-0.32*	0.24	-0.06	0.95***
TC	0.27*	-0.07	0.016	-0.021	-0.11	-0.09		0.01	0.32*	0.96***	-0.24	0.19	0.17	-0.15
TG	0.23	0.20	0.10	-0.15	0.25	0.28*	0.40**		-0.43**	-0.17	0.22	-0.10	-0.06	0.14
HDL-C	0.04	-0.07	-0.03	-0.09	-0.01	0.03	0.05	-0.27*		0.29*	-0.03	-0.06	0.06	-0.10
LDL-C	0.23	-0.07	0.10	-0.15	-0.16	-0.13	0.72***	0.17	-0.20		-0.24	0.17	0.20	-0.19
CRT	0.27	0.14	-0.01	-0.12	0.13	0.13	0.10	0.17	0.23	0.00		-0.55***	-0.29*	-0.29*
eGFR	-0.40**	-0.30*	-0.15	-0.05	0.18	0.18	-0.18	-0.19	-0.09	-0.08	-0.78***		0.34*	0.21
FBG	0.10	-0.05	-0.03	-0.04	-0.12	-0.04	0.07	-0.08	0.02	0.07	0.06	-0.07		-0.02
RBC	-0.02	0.14	-0.04	-0.27*	0.83***	0.88***	-0.08	0.21	0.07	-0.09	0.15	0.14	-0.01	

BMI=body mass index; SBP=systolic blood pressure; DBP=diastolic blood pressure; PRT=proteinuria; HGB=haemoglobin; HCT=haematocrit; TC=total cholesterol; TG=triglycerides; HDL-C=high density lipoprotein cholesterol; LDL-C=low density lipoprotein cholesterol; CRT=creatinine; eGFR=estimated glomerular filtration rate; FBG=fasting blood glucose; RBC=red blood cell. *.Correlation is significant at the 0.05 level (2-tailed), **.Correlation is significant at the 0.01 level (2-tailed), ***.Correlation is significant at the 0.001 level (2-tailed).

Table 3.13 represents the Pearson's correlation coefficients of the various parameters of the study population; there are significant positive correlations between the various haematological parameters (HGB, HCT and RBC) and between SBP and DBP (hypertension) among the control group and subjects with CKD. There was generally no significant correlation among the various haematological parameters in relation to creatinine (CRT) within the control group as opposed to the negative but significant correlation among the haematological parameters in relation to CRT among the subjects with CKD. CRT in relation to eGFR and FBG gave negative but significant correlation among the CKD subjects. Among the control group, it is only CRT in relation to eGFR that indicates such a correlation. With the exception of HDL-C in relation to AI which showed a significant negative correlation all other lipid fractions showed a significant positive correlation in relation to AI among both the control group and CKD group. eGFR also indicated a significantly positive correlation with FBG only within the CKD group as shown in Table 3.13.

Table 3.14 Odds ratio of components of cardiovascular disease among anaemic and non-anaemic CKD subjects

Parameter	Total (n=146)	CKD+Anaemia (n=78)	CKD-Anaemia (n=68)	P Value	Odds Ratio
Hypertension	45(30.8%)	32(41.02%)	13(19.1%)	0.0067	2.9
Obesity	51(34.9%)	8(10.2%)	14(20.6%)	0.1052	0.4
Hypercholesterolaemia	84(57.5%)	46(59.0%)	20(29.4%)	0.0004	2.9
Hypertriglyceridaemia	69(47.2%)	46(59.0%)	37(54.4%)	0.6178	1.2
Low HDL	135(92.4%)	42(53.8%)	33(48.5%)	0.7416	1.1
Hyperglycaemia	97(66.4%)	58(75.0%)	34(50.0%)	0.0034	2.9

Table 3.14 represents the odds ratios of components of cardiovascular disease among anaemic and non anaemic CKD subjects. The CKD patients with anaemia were 3 times at risk of developing hypertension OR=2.9; CI=1.3-6.2), hyperglycaemia (OR=2.9; CI=1.4-5.8) and hypercholesterolaemia (OR = 3.4; CI = 1.5-5.7) compared to those without anaemia (Table 3.14).

Table 3.15: Crude odds ratios of cardiovascular risk factors of study population

	CKD Subjects		Combined	
Variables	**OR(95% CI)**	**P value**	**OR(95% CI)**	**P value**
Age (continuous)	1.0(0.9 - 1.0)	0.662	1.0(0.9 - 1.0)	0.096
Age (grouped)				
(12 - 30)	1		1	
(31 - 49)	0.6(0.2 - 2.6)	0.525	1.5(0.4 - 5.1)	0.517
(50 - 68)	0.5(0.1 - 1.9)	0.301	1.6(0.5 - 5.1)	0.467
(69 - 87)	2.1(0.4 - 11.6)	0.395	3.3(0.8 - 13.7)	0.108
Sex (male)	0.9(0.4 - 2.0)	0.864	1.2(0.6 - 2.5)	0.600
TC (>5.2)	2.1(0.9 - 4.6)	**0.048**	2.8(1.3 - 5.8)	**0.008**
Triglycerides (>1.7)	1.0(0.5 - 2.1)	0.962	1.3(0.6 - 2.6)	0.476
HDL-C (<1.3)	0.4(0.2 - 1.1)	0.08	0.6(0.2 - 1.6)	0.316
LDL-C (>4.12)	1.4(0.6 - 3.4)	0.419	1.8(0.8 - 4.2)	0.155
BMI (>=30)	3.3(1.3 - 8.5)	**0.012**	1.6(1.1 - 2.4)	**0.026**
Proteinuria (>=1)	0.5(0.2 - 1.3)	0.158	0.7(0.4 - 1.1)	0.158
4v MDRD (<60)	1.0(0.5 - 2.0)	0.895	1.4(0.7 - 2.9)	0.334
FBG (>=6.1)	0.4(0.2 - 0.9)	**0.033**	0.4(0.2 - 0.9)	**0.033**
HGB (<11.0)	0.8(0.6 - 1.2)	0.323	1.3(0.7 - 2.7)	0.413

OR=odds ratio; TC=total cholesterol; HGB=haemoglobin; FBG=fasting blood glucose; BMI=body mass index; 4v MDRD=4 variable modification of diet in renal disease; LDL-C=low density lipoprotein cholesterol; HDL-C=high density lipoprotein cholesterol.

Table 3.15 represents the crude odds ratios of cardiovascular disease risk factors of study population. High TC (OR=2.1; 95% CI 0.9-4.6; p=0.048) and BMI (OR=3.3; 95% CI 1.3-8.5) were significant risk factors of cardiovascular disease in the CKD subjects.

Table 3.16: Age and sex adjusted odds ratios of cardiovascular disease risk factors of study population

	CKD Subjects		Combined	
Variables	**Adjusted OR(95% CI)**	**P value**	**Adjusted OR(95% CI)**	**P value**
Cholesterol (>5.2)	2.1(0.9 - 4.7)	**0.053**	2.8(1.3 - 5.9)	**0.009**
Triglycerides (>1.7)	0.9(0.4 - 2.0)	0.883	1.2(0.6 - 2.6)	0.558
HDL-C (<1.3)	0.4(0.2 - 1.1)	0.077	0.6(0.2 - 1.5)	0.279
LDL-C (>4.12)	1.2(0.8 - 1.9)	0.42	1.8(0.7 - 4.2)	0.208
BMI (>=30)	1.9(1.2 - 3.0)	**0.011**	2.9(1.2 - 6.9)	**0.016**
Proteinuria (>=1)	0.5(0.2 - 1.3)	0.16	0.5(0.2 - 1.3)	0.16
4v MDRD (<60)	0.9(0.4 - 2.0)	0.809	1.3(0.6 - 2.7)	0.469
FBG (>=6.1)	0.4(0.2 - 0.9)	**0.020**	0.4(0.2 - 0.9)	**0.020**
HGB (<11.0)	0.8(0.4 - 1.6)	0.481	1.4(0.7 - 2.9)	0.358

OR= odds ratio

Table 3.16 shows the age and sex adjusted odds ratios of cardiovascular disease risk factors of study population. When adjusted for age and sex, elevated TC (OR=2.1; 95% CI 0.9-4.7; p=0.053) and BMI (OR=1.9; 95% CI 1.2-3.0; p=0.011) were significantly associated with the risk of cardiovascular disease in the CKD subjects.

3.4 Oxidative Stress among Ghanaian Patients Presenting With Chronic Kidney Disease.

Table 3.17 Demographic, clinical and biochemical characteristics of study population

Parameters	Control (n=80)	CKD (n=146)	P value
Age (yrs)	46.35 ± 1.96	50.18 ± 1.14	0.072
BMI (kg m^2)	24.66 ± 0.80	24.44 ± 0.44	0.8021
SBP (mmHg)	120.7 0 ± 1.82	140.40 ± 3.84	0.0001
DBP (mmHg)	70.42 ± 1.25	90.32 ± 2.61	0.0001
PRT (g/l)	0.04 ± 0.02	1.17 ± 0.26	0.0001
HGB (g/dl)	12.45 ± 0.19	10.51 ± 0.20	<0.0001
Biochemical assays			
CRT (µmol/l)	105.90 ± 3.96	268.00 ± 25.60	0.0001
BUN (mmol/l)	3.51 ± 0.17	15.45 ± 2.80	0.0001
FBG (mmol/l)	5.31 ± 0.17	8.75 ± 0.33	0.0001
TC (mmol/l)	4.54 ± 0.13	5.63 ± 0.13	0.0274
TG (mmol/l)	1.52 ± 0.08	1.84 ± 0.09	0.0086
HDL-C (mmol/l)	1.35 ± 0.05	1.61 ± 0.20	0.2114
LDL-C (mmol/l)	2.75 ± 0.10	3.30 ± 0.14	0.0134
eGFR (ml/min/173 m^2)	92.40 ± 5.67	57.61 ± 4.15	0.0001
Oxidative stress markers			
VIT C (mg/ml)	0.54 ± 0.02	0.34 ± 0.05	0.0001
VIT A (µmol/l)	9.76 ± 3.03	16.17 ± 5.21	0.0012
MDA (µmol/l)	1.22 ± 0.10	2.66 ± 0.07	0.0001
Uric acid (µmol/l)	266.68 ± 11.00	333.90 ± 10.02	<0.0001
CAT (units/ml)	57.49 ± 1.18	71.98 ± 2.91	0.0001

BMI=Body mass index; SBP=Systolic blood pressure; DBP=Diastolic blood pressure; PRT=Proteinuria; HGB=Haemoglobin; TC=Cholesterol; HDL-C=High density lipoprotein; TG=Triglyceride; LDL-C= Low density lipoprotein; CRT = Creatinine; BUN=Blood urea nitrogen; FBG=Fasting blood glucose; eGFR=estimated glomerular filtration rate; CAT=Catalase.

Demographic, clinical and biochemical characteristics of the study population are shown in Table 3.17. The mean age of the 146 participants involved in this study was 50.18 with 45.2% of the participants being males. Apart from estimated GFR (eGFR) and haemoglobin (HGB) which were significantly decreased as compared to the control, lipid fractions (LDL-C, TC, TG) (except HDL-C), fasting blood glucose (FBG), blood pressure (SBP and DBP), creatinine (CRT), blood urea nitrogen (BUN) and proteinuria (PRT) and marker of oxidative stress (MDA) were significantly increased in the CKD subjects compared to the control group. Apart from Vit C which decreased significantly in the CKD group compared to the controls, the other antioxidants (Vit A, CAT and uric acid) showed a significant increase in CKD patients compared to controls as shown in Table 3.17.

Table 3.18 Demographic, clinical and biochemical parameters during various stages of chronic kidney disease

Parameters	CKD STAGE						
	Controls (n=80)	1 (n=25)	2 (n=35)	3 (n=37)	4 (n=25)	5 (n=24)	P Value
BMI (kg/m²)	24.66 ± 0.80	25.97 ± 0.92	25.60 ± 1.54	24.60 ± 0.98	24.11 ± 0.81	23.56 ± 0.75	0.7815
SBP (mmHg)	120.70 ± 1.82	129.1 ± 3.38*	126.00 ± 3.70	134.3 ± 3.82*	135.6 ± 4.36	136.00 ± 4.36*	0.0052
DBP (mmHg)	70.21 ± 1.26	79.2 ± 2.70***	78.92 ± 1.85*	79.43 ± 1.87*	84.20 ± 2.80	90.50 ± 4.94***	<0.0001
PRT (g/l)	0.04 ± 0.02	0.29 ± 0.06	0.82 ± 0.26	1.13 ± 0.29	1.27 ± 0.38	1.49 ± 0.40	0.0544
FBG (mmol/l)	5.31 ± 0.17	8.19 ± 0.81	8.55 ± 0.85	8.95 ± 0.70	10.66 ± 0.93*	7.28 ± 0.71	<0.0001
TC (mmol/l)	4.54 ± 0.13	5.56 ± 0.28	5.65 ± 0.25	5.75 ± 0.39	5.98 ± 0.24	6.55 ± 0.26	0.0172
TG (mmol/l)	1.52 ± 0.08	1.05 ± 0.26	1.07 ± 0.02	1.53 ± 0.37	1.83 ± 0.25	2.69 ± 0.46**	0.0013
HDL-C (mmol/l)	1.35 ± 0.05	1.73 ± 0.28	1.09 ± 0.16	1.76 ± 0.25	1.32 ± 0.16	1.23 ± 0.10	0.0660
LDL-C (mmol/l)	106.30 ± 4.00	133.60 ± 20.47	152.10 ± 14.85	159.00 ± 39.00	107.20 ± 10.81	95.00 ± 19.70	0.1423
ALB (g/l)	42.22 ± 1.01	35.55 ± 1.90**	35.75 ± 1.42**	35.63 ± 1.11***	33.54 ± 1.17***	27.69 ± 1.42***	<0.0001
Uric acid (μmol/l)	266.68 ± 11.00	255.70 ± 17.83	266.20 ± 17.12	277.70 ± 19.83	324.50 ± 29.5	309.70 ± 21.41	0.7363
MDA (μmol/l)	1.22 ± 0.10	2.41 ± 0.15*	2.45 ± 0.13**	2.59 ± 0.17**	2.67 ± 0.15***	2.87 ± 0.18***	<0.0001
CAT (units/ml)	57.49 ± 1.18	58.58 ± 2.61	59.25 ± 4.77	60.00 ± 5.00	71.63 ± 6.88	79.72 ± 8.45***	0.0001
VIT C (mg/ml)	0.54 ± 0.02	0.54 ± 0.07	0.34 ± 0.03**	0.30 ± 0.04**	0.33 ± 0.06**	0.21 ± 0.02***	<0.0001
VIT A (μmol/l)	9.76 ± 3.03	8.79 ± 1.04	9.94 ± 2.20	19.42 ± 2.10**	8.72 ± 1.08	33.98 ± 1.22***	<0.0001
e GFR(ml/min/173m²)	92.40 ± 5.67	150.50 ± 9.22***	67.47 ± 1.34	44.34 ± 1.35***	21.50 ± 0.98***	8.45 ± 0.67***	<0.0001

*BMI=Body mass index, SBP=Systolic blood pressure, DBP= Diastolic blood pressure, PRT=Proteinuria, TC= Cholesterol, HDL-C=High density lipoprotein-Cholesterol, TG=Triglyceride, LDL-C=Low density lipoprotein, GFR=Glomerular filtration rate, FBG= Fasting blood glucose, MDA= Malondialdehyde, Vit C=Vitamin C, Vit A=Vitamin A, CAT= Catalase, Alb=Albumin. Stage 1 = eGFR ≥90 mL/min/1.73m²; stage 2 = eGFR 60-89 mL/min/1.73m²; stage 3 = eGFR 30-59 mL/min/1.73m²; stage 4 = eGFR 16-29 mL/min/1.73m²; stage 5 = eGFR<15 mL/min/1.73m² or dialysis. *p<0.05, **p<0.01, ***p<0.001.*

Table 3.18 represents demographic, clinical and biochemical parameters during various stages of CKD. Apart from HGB and eGFR which decreased significantly among the CKD subjects as the condition progressed from stage 1 to 5; CRT, BUN, PRT, SBP and DBP, increased significantly at some stages of CKD. With the exception of LDL-C and HDL-C the other lipid fractions (TC and TG) increased significantly as the condition progressed with TG increasing significantly at stage 5. The marker of oxidative stress (MDA) increased significantly with the severity of CKD, whereas with the exception of Vit C which decreased as the condition progressed, the antioxidants (Vit A, CAT, uric acid) increased with the severity of the condition (Table 3.18).

Table 3.19 Pearson correlation coefficients of clinical variables and anthropometric measurement for CKD subjects

	Age	HGB	BMI	SBP	DPB	PRT	TC	TG	HDL	LDL	VLDL	BUN	CRT	eGFR	FBG	MDA	Vit C	Vit A	CAT	UA	ALB
Age		0.28	0.24	0.28	0.19	-0.16	0.18	0.17	-0.20	0.19	0.09	-0.09	-0.10	0.09	0.20	0.01	0.30*	0.24	0.16	0.26	0.15
HGB			-0.13	0.18	0.26*	0.00	-0.16	0.12	-0.10	-0.21	0.10	-0.33*	-0.25	0.20	-0.06	0.13	-0.03	0.15	0.06	0.08	0.09
BMI				0.19	0.23	0.16	0.07	-0.16	0.13	0.11	-0.24	-0.10	-0.13	0.07	0.16	-0.30*	-0.30*	0.06	-0.25*	0.08	0.13
SBP					0.76***	0.10	-0.23	-0.19	-0.37**	-0.14	-0.12	-0.04	0.05	-0.05	-0.23	0.16*	0.13	0.16	0.07	0.21	0.24
DBP						0.03	-0.21	-0.14	-0.18	-0.17	-0.15	-0.08	0.03	0.05	-0.30*	0.19*	0.09	0.19	0.16	0.15	0.20
PRT							0.16	0.13	-0.07	0.12	0.21	0.09	0.11	0.10	0.08	-0.20	-0.07	0.09	-0.12	-0.21	-0.27
TC								0.02	0.32*	0.96***	0.06	-0.23	-0.24	0.19	0.17	-0.15	-0.08	-0.10	-0.16	0.11	-0.17
TG									-0.43**	-0.17	0.86***	0.28*	0.22	-0.10	-0.06	-0.21	0.04	-0.04	-0.01	-0.27	0.17
HDL										0.28*	-0.54***	0.01	-0.03	-0.06	0.06	0.01	-0.26	-0.17	-0.08	0.02	-0.05
LDL											-0.11	-0.24	-0.24	0.17	0.20	-0.08	-0.05	-0.10	-0.12	0.19	-0.18
VLDL												0.06	0.04	-0.03	-0.08	-0.29*	0.02	0.00	-0.23	-0.18	0.04
BUN													0.91***	-0.66***	-0.27	0.24	0.33*	-0.17	0.35*	-0.18	0.10
CRT														-0.70***	-0.28*	0.17	-0.29*	0.14	0.32*	-0.13	0.05
eGFR															-0.34*	-0.31*	0.05*	-0.25*	0.08*	-0.05	-0.03
FBG																-0.11	-0.30*	0.03	-0.12	-0.07	0.04
MDA																	-0.31*	0.11	0.22	0.22	0.11
Vit C																		-0.29*	0.59***	-0.03	0.09
Vit A																			0.22	-0.08	0.20
CAT																				-0.14	-0.01
UA																					0.09
ALB																					

***.Correlation is significant at the 0.05 level (2-tailed), **.Correlation is significant at the 0.01 level (2-tailed), ***.Correlation is significant at the 0.001 level (2-tailed). BMI = Body mass index, SBP=Systolic blood pressure, DBP= Diastolic blood pressure, PRT = Proteinuria, HGB= Haemoglobin, TC= Cholesterol, HDL=High density lipoprotein, TG= Triglyceride, LDL= Low density lipoprotein, Very Low density lipoprotein, CRT = Creatinine, BUN= Blood urea nitrogen, eGFR= Estimated Glomerular filtration rate, UA= Uric acid, FBG= Glucose, MDA= Malondialdehyde, Vit C=Vitamin C, Vit A=Vitamin A, CAT= Catalase, Alb=Albumin.*

From the Pearson correlation analysis in Table 3.19, there is a significant positive correlation between blood pressure (SBP and DBP) and the marker of oxidative stress (MDA) among the CKD group. Furthermore, with the exception of vitamin C which showed a generally significant positive correlation with eGFR, the other antioxidants (Vit A, CAT and uric acid) showed a negative but significant correlation with eGFR. Again, there was significant negative correlation between FBG and MDA among CKD subjects. Furthermore, there was a significant negative correlation between eGFR and FBG and between CRT and eGFR among subjects with CKD (Table 3.19).

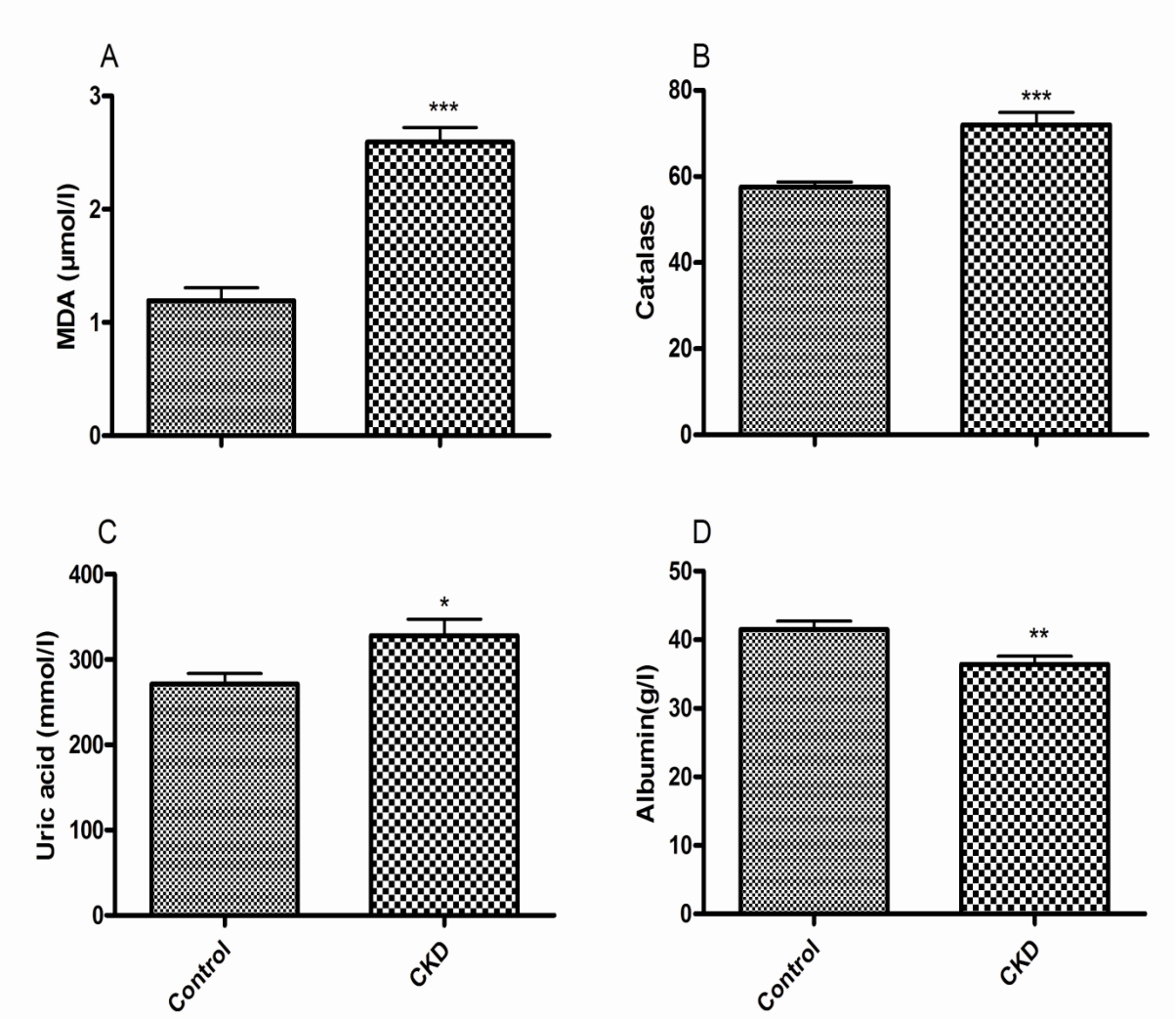

***Figure 3.7** Levels of plasma MDA (A), catalase activity (B), uric acid (C), and albumin (D) in controls and CKD patients. Results are means ± SEM. Values significantly different from controls *=p<0.05, **=p<0.01, ***p<0.001*

Figure 3.7 represents levels of plasma MDA (A), catalase activity (B), uric acid (C), and albumin (D) in controls and CKD patients. Apart from albumin (3.7D) which showed a significant decrease in the CKD subjects compared to the controls, MDA (3.7A), uric acid (3.7C) and catalase (3.7B) generally increased significantly in the CKD subjects compared to the controls.

Table 3.20 Demographic and biochemical characteristics of the study population

				Stratification of CKD subjects					
				Gender			Levels of PTH		
Parameter	Control (n=80)	Subjects (n=146)	P Value	Female (n=80)	Male (n=66)	P Value	Normal PTH (n= 20)	High PTH (n=126)	P Value
Age (years)	46.35 ± 1.96	50.18 ± 1.14	0.0720	49.46 ± 1.36	51.05 ± 1.91	0.4919	47.61 ± 3.90	50.55 ± 1.18	0.3953
SBP (mmHg)	120.70 ± 1.82	140 ± 3.84	<0.0001	133.00 ± 2.58	131.40 ± 2.78	0.6740	135.00 ± 7.58	132.00 ± 1.9	0.6130
DBP (mmHg)	70.42 ± 1.25	90.32 ± 2.61	<0.0001	81.96 ± 1.69	81.56 ± 1.80	0.8725	89.38 ± 6.22	80.83 ± 1.13	0.0281
FBG (mmol/l)	5.31 ± 0.17	8.75 ± 0.33	<0.0001	9.00 ± 0.47	8.44 ± 0.47	0.4053	9.18 ± 1.09	8.69 ± 0.35	0.6423
Na^+ (mmol/l)	141.90 ± 0.61	137.10 ± 0.48	<0.0001	137.10 ± 0.564	136.70 ± 0.85	0.6740	137.00 ± 2.15	136.90 ± 0.47	0.9590
K^+ (mmol/l)	4.25 ± 0.08	4.87 ± 0.05	<0.0001	4.72 ± 0.06	4.98 ± 0.08	0.0144	4.93 ± 0.14	4.82 ± 0.05	0.5014
Mg^{2+} (mmol/l)	0.80 ± 0.03	1.15 ± 0.03	<0.0001	1.12 ± 0.04	1.19 ± 0.05	0.2743	0.81 ± 0.04	1.20 ± 0.04	0.0002
t Ca^{2+} (mmol/l)	2.21 ± 0.03	2.04 ± 0.02	<0.0001	2.02 ± 0.02	2.07 ± 0.04	0.2797	2.22 ± 0.03	2.01 ± 0.02	0.0030
Adj Ca (mmol/l)	2.14 ± 0.05	2.12 ± 0.02	0.7211	2.12 ± 0.03	2.12 ± 0.03	0.9683	2.34 ± 0.40	2.09 ± 0.02	0.0003
PO_4^{3-} (mmol/l)	1.25 ± 0.05	2.27 ± 0.08	<0.0001	2.16 ± 0.10	2.41 ± 0.14	0.1577	1.60 ± 0.13	2.37 ± 0.09	0.0026
Na^+/K^+	33.29 ± 0.78	28.77 ± 0.32	<0.0001	29.44 ± 0.42	27.93 ± 0.49	0.0198	28.17 ± 0.89	28.85 ± 0.34	0.4873
Na^+/Mg^{2+}	203.60 ± 6.09	135.30 ± 4.15	<0.0001	139.60 ± 5.76	130.00 ± 05.94	0.2536	178.00 ± 10.70	129.20 ± 4.24	<0.0001
Na^+/Ca^{2+}	63.93 ± 1.62	68.41 ± 0.87	<0.0001	68.98 ± 1.05	67.70 ± 1.47	0.4700	61.94 ± 1.35	69.32 ± 0.95	0.0050
Ca^{2+}/K^+	0.52 ± 0.01	0.43 ± 0.01	<0.0001	0.43 ± 0.01	0.42 ± 0.01	0.5259	0.45 ± 0.01	0.42 ± 0.01	0.1604
Ca^{2+}/Mg^{2+}	3.26 ± 0.10	2.06 ± 0.07	<0.0001	2.09 ± 0.10	2.02 ± 0.11	0.6629	2.90 ± 0.12	1.94 ± 0.07	<0.0001
K^+/Mg^{2+}	6.19 ± 0.15	4.76 ± 0.15	<0.0001	4.79 ± 0.20	4.71 ± 0.22	0.7861	6.47 ± 0.48	4.51 ± 0.14	<0.0001
PTH (pg/ml)	49.33 ± 1.51	210.80 ± 13.72	<0.0001	203.50± 17.15	219.80 ± 22.27	0.5575			
e GFR (ml/min/ 1.73 m^2)	92.40 ± 5.67	57.61 ± 4.15	<0.0001	50.16 ± 4.12	66.79 ± 7.63	0.0460	134.70 ± 12.26	46.69 ± 3.46	<0.0001

SBP= Systolic blood pressure; DBP = Diastolic blood pressure; FBG= Fasting blood glucose; K^+ = Potassium; Na^+ = Sodium; Mg^{2+}= Magnesium tCa^{2+}=Total calcium; Adj Ca=Adjusted calcium; PO_4^{3-}=Phosphate; Na^+/K^+= Sodium/Potassium ratio; Na^+/Mg^{2+} =Sodium/Magnesium ratio; Na^+/Ca^{2+}=Sodium/Calcium ratio; Ca^{2+}/K^+=Calcium/Potassium ratio; Ca^{2+}/Mg^{2+}=Calcium/Magnesium ratio; K^+/Mg^{2+}=Potassium/Magnesium ratio; PTH = Parathyroid hormone; eGFR=Estimated Glomerular filtration rate; CRT = Creatinine.

Table 3.20 shows the demographic, electrolyte and electrolyte ratios of CKD subjects compared to controls. From this study, the mean ± SEM of Na^+, tCa^{2+}, Ca^{2+}/Mg^{2+} ratio, K^+/Mg^{2+} ratio, $^+$, Na^+/Mg^{2+} ratio, Na^+/K^+ ratio eGFR and Ca^{2+}/K^+ ratio were significantly lower whereas K^+, blood pressure (SBP, DBP), Mg^{2+}, PO_4^{3-}, PTH, CRT, FBG, Na^+/Ca^{2+} ratio were significantly higher in the CKD patients compared to the controls. However, the mean ages and Adj Ca were similar in both CKD subjects and controls.

When the CKD patients were stratified by gender, serum K^+, creatinine and eGFR significantly increased whereas Na^+/K^+ ratio significantly decreased in the males compared to the females. The following parameters decreased significantly in CKD subjects with elevated PTH: DBP, tCa^{2+}, Na^+/Mg^{2+}, Ca^{2+}/Mg^{2+}, K^+/Mg^{2+}, eGFR, whereas Mg^{2+} and PO_4^{3-}.

Table 3.21 Demographic and biochemical parameters during various stages of chronic kidney disease

Parameter	Controls (n=80)	CKD STAGE (ml/min/1.73m²) 1 (n=24)	2 (n=35)	3 (n=37)	4 (n=25)	5 (n=24)	P Value
K^+ (mmol/l)	4.25 ± 0.07	3.84 ± 0.08*	4.60 ± 0.09	4.71 ± 0.10**	4.80 ± 0.14**	5.15 ± 0.10***	<0.0001
Na^+ (mmol/l)	142.00 ± 0.61	136.30 ± 1.67***	138.10 ± 0.93*	136.20 ± 0.66***	137.50 ± 1.11*	135.30 ± 1.06*	<0.0001
Mg^{2+} (mmol/l)	0.80 ± 0.02	0.81 ± 0.03	0.91 ± 0.04	1.25 ± 0.06**	2.35 ± 0.21***	4.60 ± 0.22***	<0.0001
PO_4^{3-} (mmol/l)	1.25 ± 0.05	1.53 ± 0.06	1.39 ± 0.06	2.00 ± 0.09***	2.98 ± 0.10***	3.92 ± 0.10***	<0.0001
t Ca^{2+} (mmol/l)	2.21 ± 0.03	2.19 ± 0.03	2.18 ± 0.04	2.08 ± 0.03	1.88 ± 0.04***	1.78 ± 0.08***	<0.0001
Adj Ca (mmol/l)	2.14 ± 0.05	2.28 ± 0.04	2.27 ± 0.04	2.17 ± 0.04	2.00 ± 0.03	1.78 ± 0.04***	<0.0001
Ca^{2+}/Mg^{2+}	2.91 ± 0.11	3.12 ± 0.20	2.62 ± 0.13	1.92 ± 0.14***	1.10 ± 0.05***	0.89 ± 0.06***	<0.0001
K^+/Mg^{2+}	6.19 ± 0.153	6.13 ± 0.38	5.51 ± 0.27	4.25 ± 0.28***	3.54 ± 0.21***	3.54 ± 0.21***	<0.0001
Na^+/K^+	33.29 ± 0.78	28.46 ± 0.96	29.74 ± 0.62***	29.32 ± 0.64***	28.76 ± 0.77***	26.80 ± 0.54***	<0.0001
Na^+/Ca^{2+}	63.93 ± 1.62	62.59 ± 1.54	63.80 ± 1.23	65.78 ± 0.86	74.11 ± 1.93***	79.05 ± 2.81***	<0.0001
Na^+/Mg^{2+}	203.60 ± 6.09	174.70 ± 8.56	163.00 ± 7.86***	122.90 ± 8.06***	116.20 ± 6.13***	94.41 ± 5.66	<0.0001
Ca^{2+}/K^+	0.53 ± 0.01	0.45 ± 0.10**	0.47 ± 0.01**	0.44 ± 0.01***	0.39 ± 0.01***	0.35 ± 0.01***	<0.0001
PTH (pg/ml)	49.33 ± 1.51	69.03 ± 2.21	95.23 ± 3.14***	141.10 ± 7.43***	327.20 ± 14.91***	515.80 ± 7.20***	<0.0001
FBG (mmol/l)	5.31 ± 0.17	8.19 ± 0.81	8.55 ± 0.85	8.95 ± 0.70	10.66 ± 0.93*	7.28 ± 0.71	<0.0001
SBP (mmHg)	120.70 ± 1.82	129.1 ± 3.38*	126.00 ± 3.70	134.3 ± 3.82*	135.6 ± 4.36	136.00 ± 4.36*	0.005
DBP (mmHg)	70.21 ± 1.26	79.2 ± 2.70***	78.92 ± 1.85*	79.43 ± 1.87*	84.20 ± 2.80	90.50 ± 4.94***	<0.0001

**p<0.05, **p<0.01, ***p<0.001. CKD=Chronic kidney disease; SBP= Systolic blood pressure; DBP = Diastolic blood pressure; FBG= Fasting blood glucose; K^+ = Potassium; Na^+ = Sodium; Mg^{2+}= Magnesium tCa^{2+}=Total calcium; Adj Ca=Adjusted calcium; PO_4^{3-}=Phosphate; Na^+/K^+= Sodium/Potassium ratio; Na^+/Mg^{2+} =Sodium/Magnesium ratio; Na^+/Ca^{2+}=Sodium/Calcium ratio; Ca^{2+}/K^+=Calcium/Potassium ratio; Ca^{2+}/Mg^{2+}=Calcium/Magnesium ratio; K^+/Mg^{2+}=Potassium/Magnesium ratio; PTH = Parathyroid hormone. Stage 1 = eGFR ≥90 mL/min/1.73m²; stage 2 = eGFR 60-89 mL/min/1.73m²; stage 3 = eGFR 30-59 mL/min/1.73m²; stage 4 = eGFR 16-29 mL/min/1.73m²; stage 5 = eGFR<15 mL/min/1.73m² or dialysis.*

Table 3.21 represents the demographic and biochemical parameters during various stages of CKD. Serum K^+, Mg^{2+} and PTH levels increased from stages 1 to 5. The increases reached statistical significance at stage 1 and stages 3 to 5; stages 3 to 5, and stages 2 to 5 for K^+, Mg^{2+} and PTH respectively. Serum phosphate decreased initially at stage 2, and increased reaching statistical significance at stages 3 to 5. Na^+, SBP and DBP decreased from stages 1 to 2 and increased afterwards. The decrease reaches statistical significance between stages 2 to 5; stages 3 and 5 for Na^+ and; SBP and DBP respectively. Adj Ca decreased from stage 1 to 5 reaching statistical significance at stages 4 and 5 respectively. Serum Ca^{2+}/K^+ ratio and Na^+/K^+ ratio levels increased significantly from stages 1 to 2 and decreased significantly afterwards. Ca^{2+}/Mg^{2+} ratio, Na^+/Mg^{2+} ratio, and K^+/Mg^{2+} ratio decreased from stages 1 to 5, reaching statistical significance at stages 3 to 5 for Ca^{2+}/Mg^{2+} ratio and K^+/Mg^{2+} ratio; and stages 2 to 4 for Na^+/Mg^{2+} ratio respectively (Table 3.21).

Table 3.22 Odds ratios of high and low levels of electrolytes among controls and CKD subjects.

Parameter	Control (n=80)	Subjects (n=146)	OR (95% CI)	P Value
High Electrolytes				
Hypernatraemia	17/80 (21.25%)	10/146 (6.85%)	0.27 (0.12-0.63)	0.0023
Hyperkalaemia	5/80 (6.25%)	56/146 (38.35%)	9.33 (3.55-24.5)	<0.0001
Hypermagnesaemia	4/80 (5.00%)	96/146 (65.75%)	36.48 (12.61-105.50)	<0.0001
Hypercalcaemia	5/80 (6.25%)	8/146 (5.48%)	0.87 (0.27-2.75)	0.7746
Hyperphosphataemia	7/80 (8.75%)	108/146 (74.00%)	29.64 (12.55-70.00)	<0.0001
Low Electrolytes				
Hyponatraemia	5/80 (6.25%)	55/146 (37.67%)	9.06 (3.45-23.81)	<0.0001
Hypokalaemia	1/80 (1.25%)	0/146 (0.00%)	0.18 (0.01-4.50)	0.354
Hypomagnesaemia	1/80 (1.25)	0/146 (0.00%)	0.18 (0.01-4.50)	0.354
Hypocalcaemia	10/80 (12.50%)	77/146 (52.74%)	7.8 (3.73-16.34)	<0.0001
Hypophosphataemia	3/80 (3.75%)	0/146 (0.00%)	0.07 (0.00-1.48)	0.0433

OR = Odds ratio;

Table 3.22 represents odds ratios of high and low levels of electrolytes among controls and CKD subjects. The risk of developing hypermagnesaemia, hyperkalaemia and hyperphosphataemia was 36 folds (OR = 36.5; 95% CI =12.6-105.5), 9 times (OR = 9.3; 95% CI = 3.5 -24.5) and about 30 times (OR = 29.6; 95% CI = 12.5-70.0) high in the CKD subjects compared to the controls. However, hypernatraemia was less likely in the CKD patients compared to the controls (OR = 0.3; 95% CI = 0.1-0.6).

Furthermore, the risk of hyponatraemia (OR = 9.06; 95% CI = 3.4-23.8) and hypocalcaemia (OR = 7.8; 95% CI = 3.7-16.3) were 9 and 8 times more pronounced in the CKD subjects compared to the controls. Conversely, the risk of hypophosphataemia was 14 times more pronounced in the controls compared to the CKD subjects (OR = 0.1; 95% CI = 0.0-1.5).

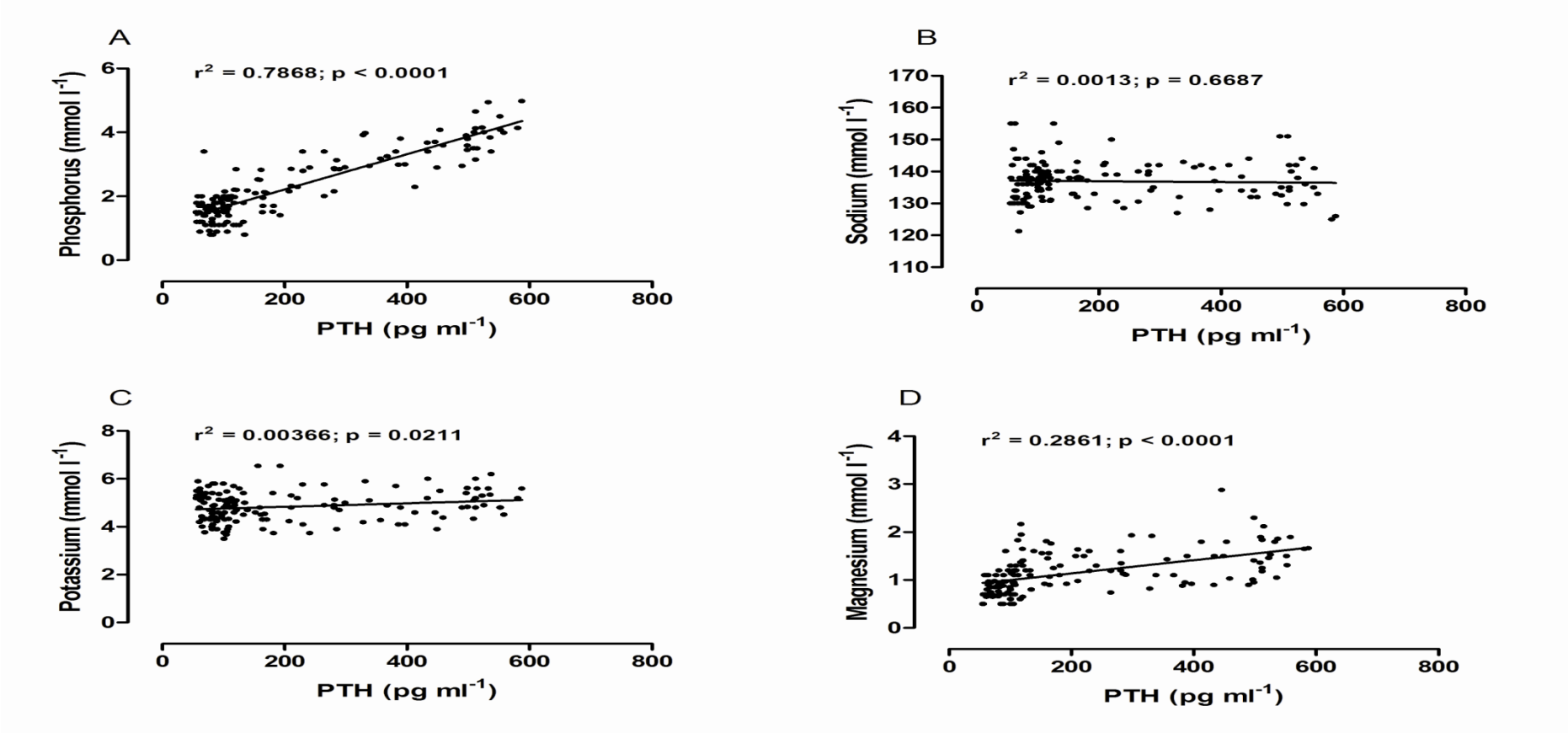

Figure 3.8 Linear regression graphs of phosphate (A), sodium (B), potassium (C), and magnesium (D) against parathyroid hormone (PTH).

Figures 3.8-3.10 show a linear regression analysis of the various electrolytes and their ratios in relation to PTH. For every mmol/l increase in the serum concentration of PO_4^{2-} ($r^2 = 0.78$, $p < 0.0001$) (Figure 3.8A), K^+ ($r^2 = 0.28$, $p < 0.0001$) (Figure 3.8C) and Mg^{2+} ($r^2 = 0.004$, $p = 0.0211$) (Figure 3.8D) there was a corresponding increase in serum concentration of PTH with beta values of 0.005, 0.0007 and 0.001, respectively. However, there was no linear relationship between Na^+ and PTH ($r^2 = 0.001$, $p = 0.6687$) (Figure 3.8B).

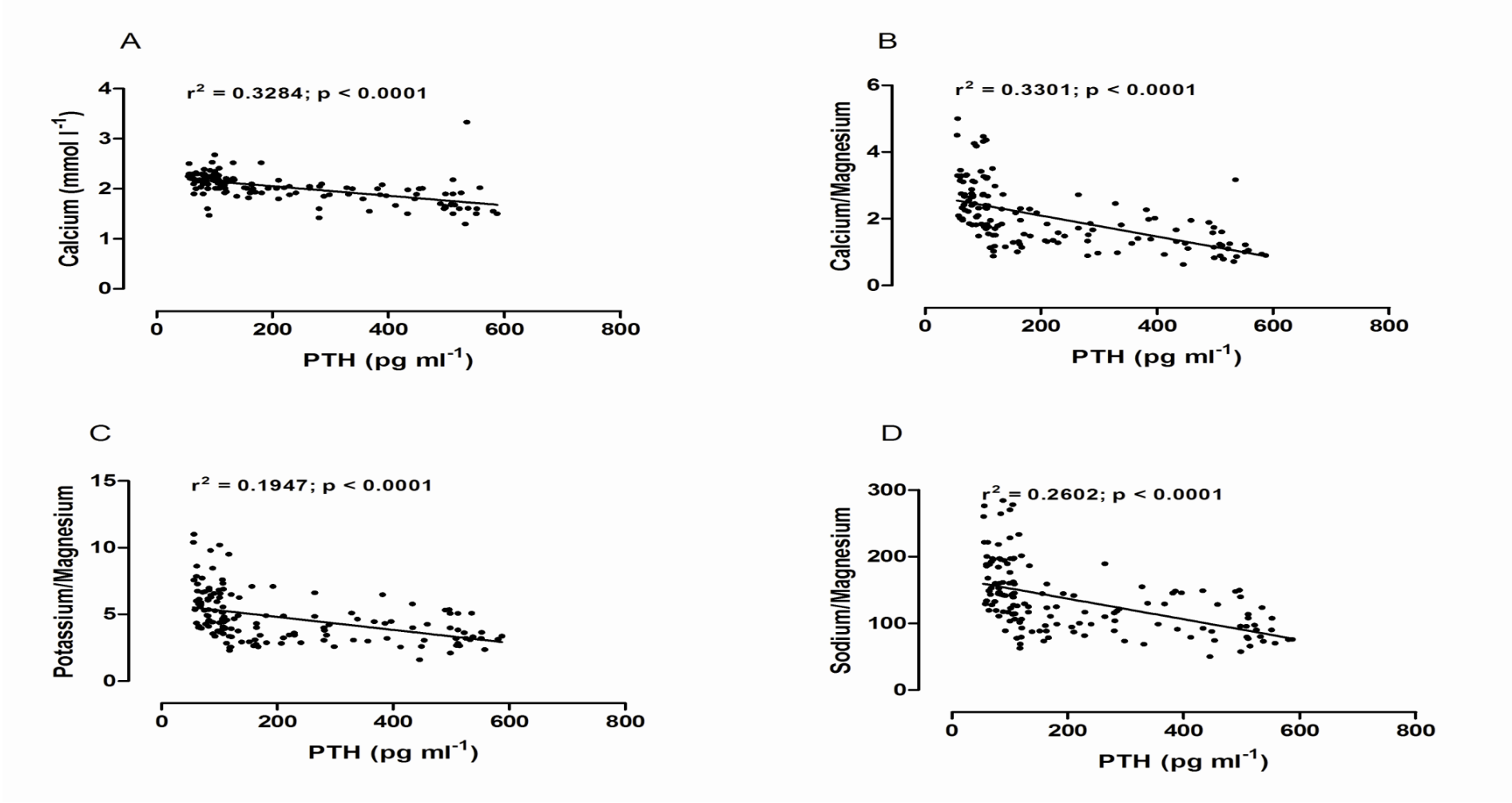

Figure 3.9 Linear regression of calcium (A), calcium/magnesium (B), potassium/magnesium (C) and sodium/magnesium (D) against parathyroid hormone (PTH).

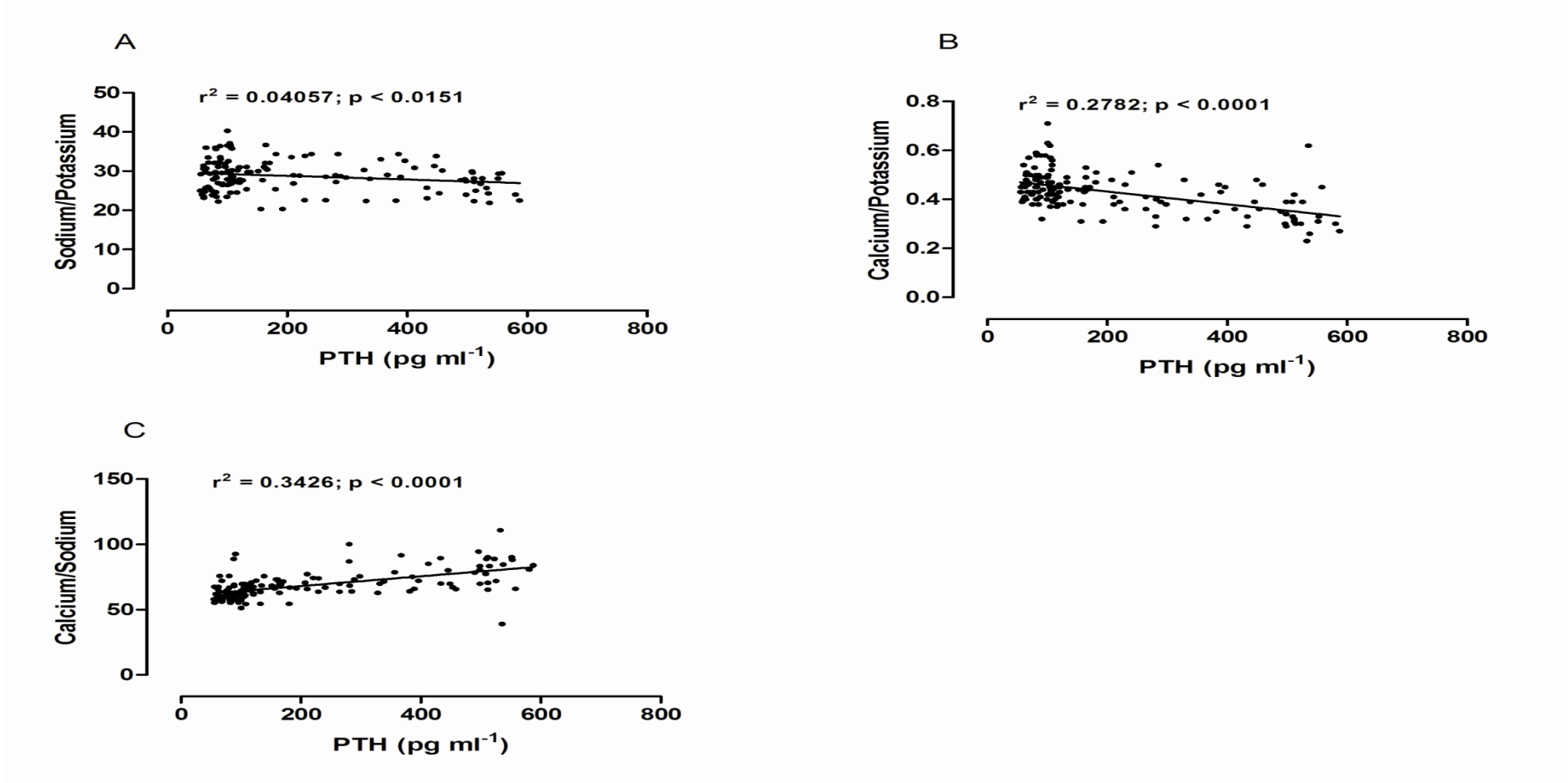

Figure 3.10 Linear regression of Sodium/Potassium (A), Calcium/Potassium (B), Calcium/Sodium (C) against parathyroid hormone (PTH).

Conversely, there was a corresponding decrease in the serum concentration of PTH, for every mmol/l increase in the serum concentrations of Ca^{2+} (r=0.33, $p < 0.0001$) (Figure 3.9 A), Ca^{2+}/Mg^{2+} ratio ($r^2 = 0.33$, $p < 0.0001$) (Figure 3.9 B), Na^{+}/Mg^{2+} ratio ($r^2 = 0.26$, $p < 0.0001$) (Figure 3.9 C) and K^{+}/Mg^{2+} (r=0.19, $p< 0.0001$) (Figure 3.9 C) with beta values of -0.001, -0.003, -0.005 and -0.154 respectively.

Furthermore, as shown in Figure 3.10 A-C, there was an inverse relationship between Na^{+}/K^{+} ratio ($r^2 = 0.04$, $p< 0.0151$), Ca^{2+}/K^{+} ratio ($r^2 = 0.28$, $p < 0.0001$) and PTH with beta values of -0.005 and -0.0002 whereas for every mmol/l increase in Ca^{2+}/Na^{+} ratio ($\beta = 0.0373$, $r^2 = 0.34$, $p < 0.0001$) there was an increase in PTH.

Chapter 4

DISCUSSION

4.1 PREDICTIVE PERFORMANCE OF RENAL FUNCTION EQUATIONS AMONG GHANAIANS PRESENTING WITH CHRONIC KIDNEY DISEASE

The similarity in age of the CKD group relative to the control group is contrary to observations made in earlier studies which have established that the risk of CKD increases as one grows older (Coresh *et al.*, 2002; National Kidney Foundation, 2002). This observation confirmed a significant trend of decreasing estimated GFR with increasing age. Apart from that, the result of this study shows a significant decrease in plasma albumin with concomitant increase in proteinuria (Table 3.2). This is in line with the work of Levey *et al*., (2003) which indicated that persistently increased proteinuria is mostly a marker of kidney damage. Usually, an untectable amount of protein is excreted in the urine of physiologically normal persons. Excretion of albumin in large quantities has been identified as a sensitive marker for CKD attributable to diabetes, glomerular disease, and hypertension. Proteinuria implies that there is an increased excretion of albumin or some other identifiable protein in the urine (Levey *et al*., 2003).

In this study, we evaluated the performances of the seven renal function formulae for estimating GFR in 146 subjects with CKD. These formulae are commonly used in daily clinical practice elsewhere and decisions regarding the care of CKD patients are based on the estimated GFR, but their accuracy is still disputable (National Kidney Foundation, 2002). Furthermore, none of these seven equations has been validated among Africans and for that matter Ghanaian adult CKD subjects. However, K/DOQI recommends the use of the CKD-EPI, 4v-MDRD and the CG equations which are presumed to be the most accurate. Accurate assessment of kidney function among patients with CKD is essential for diagnostic and interventional purposes, adequate therapeutic management, interpretation of symptoms that might be uraemic in nature, and deciding when is the appropriate time to initiate dialysis. Because of the numerous disadvantages of using creatinine

clearance and other markers, predictive equations are typically used, to estimate renal function (Jelliffe and Jelliffe, 1972; Cockcroft and Gault, 1976; Gates, 1985; Levey *et al.*, 1999a; Levey *et al.*, 1999b; Levey *et al.*, 2009).

Our results show that among the seven equations the most accurate renal function estimates were derived by using the CKD-EPI and 4v-MDRD equations with the CKD–EPI having a slight edge over the 4v-MDRD in terms of specificity thus making it more accurate which is in conformity with the work of Levey *et al.*, (2009). The CKD-EPI and 4v-MDRD equations gave sensitivity and specificity of 66.0% and 70.0%, respectively and 67.30% and 63.9%, respectively to detect GFR values less 60 ml/min/1.73 m^2.

Analysis of bias, a measure of systematic error, generally showed a very good global agreement between the renal function equations. A similar bias of -3.10 was observed when the CKD-EPI formula was compared with the GFR estimated by 4v-MDRD. In contrast, to the CKD–EPI and 4v-MDRD, the JL 1 and Gates formula were shown largely to underestimate measured GFR. It may not be clear what the inconsistencies are, but it may be down to variations in patient characteristics and may warrant further investigation.

Analysis of the ability of a formula to classify patients into different subgroups depends on the characteristics of the population. In particular, it depends on the proportion of patients who happen to be near the boundaries of the subgroups. In our study, analysis of the performance of all the formulae to classify patients according to the K/DOQI CKD classification showed that, all the formulae classified approximately the same percentage of subjects into the severe stage (stage 4) and end stage renal disease (stage 5) (Table 3.2). Though there are greater variations as one moves closer to stage 1, CKD-EPI and the 4v-MDRD either have similar mean values or similar percentage values thus confirming their superiority over the other equations and also highlights the limitations of the other formulae. Although the agreement between the 4v- MDRD and CG equation was good,

CKD-EPI was more accurate compared to 4v-MDRD particularly at more advanced stages of CKD. This observation is in conformity with other reports elsewhere (Fontsere *et al.*, 2006; Levey *et al.*, 2009). The performance of the MDRD equation improves as GFR declines.

The most important practical utility of a GFR predictive formula is to diagnose and stratify chronic kidney disease in patients with kidney disease. According to Levey *et al.,* (1999a; 2009) the CKD-EPI and the 4v- MDRD equations show better diagnostic accuracy than the CG formula as they do not require body weight. This, however, appears not to be the case in our study where the CG appeared to have a slight edge over the 4v- MDRD and CKD-EPI with a specificity of 80.0% against 67.3% and 70.0% to detect GFR values less than 60 ml/min/1.73 m^2. The 4v-MDRD formula in comparison to the CKD-EPI seems to overestimate low GFR, because the relationship between serum creatinine and GFR in severe kidney disease is not simple as the clearance of creatinine does not depend solely on GFR (Perrone *et al.,* 1992). In our study the 4v-MDRD stratified 33.7% of the subjects in stages 4 and 5 compared to the CKD-EPI equation which stratified 33.6% of the subjects in stages 4 and 5, CG however stratified 36.3%.

4.2 METABOLIC SYNDROME AMONG GHANAIAN PATIENTS PRESENTING WITH CHRONIC KIDNEY DISEASE.

The similarity within the age of the CKD group relative to the control group is contrary to observations made in earlier studies by Coresh *et al.,* (2008) which indicated that chronic kidney disease (CKD) is prevalent among the elderly and is associated with increased cardiovascular mortality. As shown in Table 3.5, the CKD subjects had proteinuria, with high blood pressure and hyperglycaemia. Proteinuria is a sign of increased protein excretion by the kidneys and hence a traditional marker of declining kidney function (Levey *et al.*, 2003). In addition to predicting loss of renal function, proteinuria has been linked with CVD and

mortality risk in population-based studies (Arnlov *et al.*, 2005). Presence of even minimal albuminuria could reflect generalized endothelial dysfunction in capillaries (e.g. glomeruli) and arteries or abnormalities within the fibrinolytic and coagulation pathways; it may be a marker of inflammatory status and may denote greater severity of end-organ injury or mark the depletion of certain antioxidants (Ford *et al.*, 2005). Moreover, in subjects older than fifty years, the proportion of sclerotic glomeruli is increased, with a spread of 0.5–36% (Kappel and Olsen, 1980) as a result of glomerular ischemia secondary to the changes in renal blood flow that occur with aging (McLachlan *et al.*, 1977). These changes resulted in not only progressive renal dysfunction but also proteinuria due to glomerular hypertension and hyperfiltration of residual glomeruli.

The increased waist circumference (WC) observed when the CKD subjects were compared to the controls is similar to the work of Pinto-Sietsma *et al.*, (2003). Indeed, central obesity, as evaluated by the waist circumference, has been indicated as an in dependent risk factor for renal dysfunction (Chen *et al.*, 2005; Kurella *et al.*, 2005; Locatelli *et al.*, 2006). Additionally, it has been demonstrated that increased body mass increases the risk for developing end-stage kidney disease (ESKD), even when adjustments are made for hypertension, proteinuria and other generally associated conditions (Iseki *et al.*, 2004; Hsu *et al.*, 2006). The major pathophysiological processes (regardless of the presence of hypertension or diabetes) embrace increased glomerular filtration rate (GFR) and changed renal haemodynamics, inflammatory and oxidative variations, extra renal sodium reabsorption and stimulation of the renin–angiotensin and sympathetic nervous systems (Locatelli *et al.*, 2006). These abnormalities result in the so-called obesity-related glomerulopathy consisting of glomerulomegaly and focal segmental glomerulosclerosis (FSGS) (Sedor and Schelling, 2005; Locatelli *et al.*, 2006). The

United States Renal Data System (USRDS), (Chan *et al.*, 2007b), has indicated that hyperglycaemia (Diabetes) is frequently linked with CKD, thus for almost 50% of patients on any form of dialysis, the primary cause of their kidney failure is diabetes. Observations made in this study demonstrate that MetS, as outlined by the NCEP ATP III criteria, was about 26.35% higher in CKD patients and is a lot common in older individuals as confirmed earlier by the works of Johnson *et al.*, (2007) and Chen *et al.,* (2004) when the CKD patients were compared to the controls. Furthermore, when the CKD patients were stratified based on the presence or absence of the MetS, CKD patients with MetS were much older as observed in earlier studies (Reynolds and He, 2005; Johnson *et al.*, 2007). Dyslipidaemia has been identified as a vital factor that not only plays a role in the initiation of CKD but also contributes to the progression of the condition (Hunsicker *et al.*, 1997; Muntner *et al.*, 2000) . TG and low HDL cholesterol has been identified as independent risk factors for CKD progression (Fried *et al.*, 2001). However, in this cohort increased TG but not low HDL-C was predictive of CKD development as observed in earlier studies by Luk *et al.,* (2008). The processes underlying the role of lipids in the initiation of renal injury have not been fully elucidated. However, several cytokines could be involved in renal injury. The exposure of the mesangial cells to lipids enhances their secretion of interleukin-6, platelet-derived growth factor, transforming growth factor-b, and tumour necrosis factor-a (Fried *et al.*, 2001). In the mesangial cells, the TG-rich lipoproteins stimulate the production and expression of fibronectin and monocyte chemoattractant protein-1 (Nishida *et al.*, 1997). Insulin-like growth factor-1 induces lipid accumulation in the mesangial cells, and this restricts their ability to respond to specific migratory and contractile stimuli (Berfield *et al.*, 2002).

Previous studies by Lloyd-Jones *et al.,* (2000) documented a high prevalence of isolated systolic hypertension (systolic pressure >140 mm Hg, with diastolic <90 mm Hg) in the general population, and data from clinical trials have documented

beneficial effects from treating isolated systolic hypertension in the elderly (Savage *et al.*, 1998). High systolic pressure is prevalent in CKD as observed among the CKD subjects with MetS in this study and is a determinant of CKD progression (Young *et al.*, 2002) and therefore systolic blood pressure control should be the focus of antihypertensive therapy in CKD. The association of CKD with isolated systolic hypertension (and wide pulse pressure) may be explained by increased vascular stiffness. Wide pulse pressure appears to be a marker of vascular stiffness and cardiovascular calcification, a predictor of cardiovascular risk in the elderly (Bielak *et al.*, 2004) and it is associated with increased mortality in patients with renal disease (Klassen *et al.*, 2002).

The current study observed a high prevalence of MetS in females compared to males. This is consistent with observations made in numerous including the Virgem das Graças community study (Dallongeville *et al.*, 2004).

This difference could be due to the high frequency of abdominal obesity among women which corroborates the hypothesis that metabolic disorders are more prevalent in females than males, and that high body weight and large waist circumference are the major contributing factors (Amoah, 2003; Dallongeville *et al.*, 2004; Owiredu *et al.*, 2008; Titty *et al.*, 2008). The decreased eGFR seen among the female CKD patients with MetS could be due to impaired endothelial function and is influenced by the numerous components of the MetS (Barylski *et al.*, 2008).

Diabetes and hypertension has been identified by epidemiological studies as the key factors associated with the development and progression of CKD (Humphrey *et al.*, 1989; Whelton *et al.*, 1996). The results of this study are in keeping with those from Western countries, intimating that even slightly increased BP (≥130/≥85 mm Hg) or slightly elevated FBG levels (≥6.1 mmol/L) are linked with an increased risk for CKD. Besides, an association between elevated TG levels and an increased risk of CKD was observed in this study, with an odds ratio over that for the association

between CKD and elevated BP or increased FBG levels as described by Zhang *et al.*, (2007) in their epidemiological study.

The 9- fold risk of diabetes, and hypertension, about 5- fold risk of low HDL-C, and 2 times risk of hypertriglyceridaemia demonstrated in this study confirmed the works of Després *et al.,* (2008) and Sarafidis (2008) who intimated that several factors including obesity (especially abdominal obesity), diabetes mellitus, hypertension and dyslipidaemia are associated with increased risk of developing CKD.

This report is in agreement with the work of Lea *et al.*, (2008) which identified proteinuria as a predictor of CKD progression in subjects with or without metabolic syndrome. This is not surprising because even microalbuminuria has been shown to cluster with metabolic syndrome, and proteinuria is a well-established predictor of CKD progression (Kovacic *et al.*, 2008).

There appears to be a graded correlation between the number of components of the MetS and the risk of CKD. After adjustments for age and gender, high SBP, increased WC and BMI, high TG and a low HDL-cholesterol level were independent predictors of renal function impairment. These findings suggest that the MetS directly contributes to the increased risk of CKD similar to studies conducted in the United States (Chen *et al.*, 2004; Kurella *et al.*, 2005).

The possible reasons for the mechanism underlying the relationship between the MetS and CKD are insulin resistance and compensatory hyperinsulinaemia. These mechanisms could directly contribute to the development of renal injury by worsening renal haemodynamics through multiple processes, including sodium retention (DeFronzo *et al.*, 1975), activation of the sympathetic nervous system (Rowe *et al.*, 1981), decreased Na^+, K^+ -ATPase activity (Clausen and Everts, 1989) and elevation of glomerular filtration fraction (Andronico *et al.*, 2002).

The relationship between the MetS and the incidence of CKD is that MetS components directly harm the kidneys through systemic atherosclerosis.

Individual components of metabolic syndrome, including glucose intolerance, hypertension, and dyslipidaemia, could act directly as risk factors for renal injury through renal or systemic atherosclerosis according to previous epidemiological studies (Humphrey *et al.*, 1989; Whelton *et al.*, 1996; Hunsicker *et al.*, 1997). In the present study, it was found that clusters of these risk factors had a stronger impact on the development of CKD than individual risk factors. Additionally, the accumulation of 3 or more of the metabolic disorders outlined by NCEP criteria promoted the development of CKD or progression of GFR decline. These findings support the hypothesis that clusters of atherogenic metabolic disorders induce renal vessel injury, resulting in deterioration of renal function (Ninomiya *et al.*, 2006).

4.3 ANAEMIA AS A RISK FACTOR FOR CARDIOVASCULAR DISEASE IN PATIENTS WITH CHRONIC KIDNEY DISEASE

In this study, the CKD subjects were more anaemic and had reduced estimated GFR (eGFR) compared to the controls (Table 3.9). This is in conformity with the findings of other studies (Coresh *et al.*, 2002; National Kidney Foundation, 2002). These parameters together provided a greater cardiovascular (CVD) risk among the CKD study population compared to the controls. These findings are consistent with recent data which showed that CVD is independently associated with kidney function decline (Elsayed *et al.*, 2007). As seen in Table 3.9, the CKD subjects had proteinuria which is an indication of increased protein excretion and hence a classical marker of declining kidney function (Levey *et al.*, 2003). Physiologically normal persons usually excrete routinely undetectable amounts of protein in the urine. Increased excretion of albumin is a sensitive marker for chronic kidney disease and may be due to diabetes, glomerular disease, and hypertension. Reduced kidney function is also associated with increased levels of inflammatory

factors (Hsu *et al.*, 2002; Shlipak *et al.*, 2003; Muntner *et al.*, 2004); abnormal apolipoprotein levels (Muntner *et al.*, 2004); elevated plasma homocysteine (Shlipak *et al.*, 2003; Muntner *et al.*, 2004); enhanced coagulability (Shlipak *et al.*, 2003); anaemia (Hsu *et al.*, 2002); left ventricular hypertrophy (Levin *et al.*, 1999); increased arterial calcification (Raggi *et al.*, 2002); endothelial dysfunction (Blacher *et al.*, 2003) and arterial stiffness (London *et al.*, 2003). How these and other factors interact to increase the risk of adverse outcomes remains unclear but are the focus of ongoing investigations (Muntner *et al.*, 2004).

When CKD subjects were stratified according to the absence or presence of anaemia, those with anaemia had a higher prevalence of several cardiovascular risk factors including older age, male gender, diabetes, and hypertension and reduced kidney function (low eGFR). This is consistent with the findings of Thomas *et al.*, (2005) who stated that the risk of cardiovascular disease in patients with moderate to severe renal impairment may be attributed, in part, to the high burden of traditional risk factors (such as diabetes, hypertension, and dyslipidaemia) in this population. However, recent evidence suggests that anaemia may also represent a significant additional risk for cardiovascular disease in patients with CKD (Tsuruya and Hirakata, 2008). Certainly, anaemia in CKD identifies patients at increased risk for hospitalization and premature death. Furthermore, these subjects had higher total cholesterol and triglyceride levels with normal values for the other lipid fractions. The high serum triglycerides observed among this cohort is partly consistent with the findings of Locatelli *et al.*, (2003c) who observed that CKD-related lipid disorders mainly consist of increased serum triglyceride levels (due to an enhanced production and accumulation of triglyceride rich lipoproteins, such as very low-density lipoproteins and intermediate-density lipoproteins due to low clearance). However, Locatelli *et al.*, (2003c) also observed low high density lipoprotein cholesterol levels and increased amounts of small low-density lipoproteins which are inconsistent with the observations made in this study. These cholesterol fractions activate

proinflammatory pathways, thereby promoting artherogenesis and endothelial dysfunction (Snively and Gutierrez, 2004).

The principal finding of this study is that anaemia and elevated serum creatinine (Scr) could confer a risk of developing adverse CVD as they modified the predisposing factors in participants who have CKD. The combination of anaemia and CKD confers a particularly high-risk group for adverse outcomes. Several, but not all, studies have suggested that anaemia may be a risk factor for adverse outcomes in different populations and that the risk may be modified by the presence of CKD (Al-Ahmad *et al.*, 2001; Abramson *et al.*, 2003; Jurkovitz *et al.*, 2003). For example, in atherosclerosis risk in communities (ARIC), anaemia was an independent risk factor for CVD outcomes (Sarnak *et al.*, 2002), and the combination of anaemia and CKD conferred a synergistic risk for cardiovascular risk factors compared with each risk factor alone (Abramson *et al.*, 2003; Jurkovitz *et al.*, 2003) as confirmed by this study (Table 3.12). Similarly, in a secondary analysis of the Studies of Left Ventricular Dysfunction (SOLVD), a randomized controlled trial that enrolled patients with an LV ejection fraction ≤35%, lower GFR, and lower HCT were independent risk factors for all-cause mortality; however, the combination conferred a synergistic risk (Al-Ahmad *et al.*, 2001).

The association between increased risk of CHD and high Scr in patients with anaemia might be explained by impairment in the physiologic mechanisms of adaptation to maintain the oxygen supply to the tissues in the presence of anaemia. These mechanisms of adaptation are both non-hemodynamic and hemodynamic (Metivier *et al.*, 2000). Non-hemodynamic mechanisms include increased erythropoietin production to stimulate erythropoiesis and increased oxygen extraction. In normal resting conditions, the non-hemodynamic factors can almost entirely compensate for the haemoglobin (HGB) deficit (Metivier *et al.*, 2000). However, in the setting of kidney disease, erythropoietin production is impaired, and, therefore, the only non-hemodynamic mechanism of compensation is an increase in oxygen extraction, which has a limited effect (London, 2001).

Besides that, a strong association has been established between anaemia, CKD and cardiovascular disease through both direct and indirect effects on the heart, leading to impaired left ventricular (LV) function, LV dilatation, heart failure and death (Shulman *et al.*, 1989; Culleton *et al.*, 1999). It is widely known that patients with a GFR < 60 ml/min per 1.73 m^2 are much more likely to have anaemia (Astor *et al.*, 2002) and the prevalence and severity of anaemia increase with declining renal function (Astor *et al.*, 2002) as confirmed by this study. Anaemia, together with the hypertension, which was also common in the CKD subjects (Table 3.12), are both known as traditional risk factors for CVD.

When subjects with only CKD were compared to the reference group (Table 3.12); the subjects were at risk of developing hypertriglyceridaemia, low HDL-C, diabetes, renal insufficiency and less likely to develop obesity. This is in agreement with the work of Muntner *et al.,* (2004) and Longenecker *et al*., (2002) whose studies showed that patients with CKD were more likely to have elevated triglyceride values and lower HDL-C values. However, on multivariate analysis obesity and high cholesterol (Table 15 and Table 16) increased the cardiovascular disease risk of the CKD subjects in both crude and age and sex controlled analysis a finding consistent with observations made in other studies (Muntner *et al.*, 2002).

4.4 OXIDATIVE STRESS AMONG GHANAIAN PATIENTS PRESENTING WITH CHRONIC KIDNEY DISEASE.

Abnormalities in lipid metabolism are common in early as well as advanced stages of kidney disease. These abnormalities have been reported to be characterized by low plasma concentrations of HDL-C, high concentrations of TG, LDL-C particles, and TC (de Sain-van der Velden *et al.*, 1998; Vaziri, 2006) as confirmed by this study except for HDL-C which was not significantly different from the controls. However, the level of HDL in CKD is inconsistent as some researchers have shown that it can either decrease (Vaziri, 2006) or remain unchanged (Shah *et al.*, 1994). The alteration in lipid metabolism has been proposed to accelerate the progression of CKD through various mechanisms. First and foremost, tubular epithelial cells

reabsorb filtered proteins (mostly lipoproteins and albumin) containing fatty acids, phospholipids, and cholesterol which then stimulate inflammation of the tubulointerstitial cells, foam cell formation, and subsequently tissue injury (Magil, 1999). Secondly, lipoproteins accumulate in the glomerular mesangium and promote the formation of matrix and glomerulosclerosis (Wheeler and Chana, 1993). Local and oxidized lipoproteins, mainly LDL, trigger the generation of matrix proteins through cultured mesangial cells and stimulate the production of proinflammatory cytokines, which initiate the recruitment and activation of resident and circulating macrophages (Rovin and Tan, 1993).

As a significant risk factor for the development of cardiovascular events in patients with CKD, dyslipidaemia requires intervention through the use of statins to avoid or minimize the sequel of these complications (Kasiske, 2003). The effects of dyslipidaemia on the kidney are mainly observed in those with hypertension, diabetes and proteinuria which are known elements of danger for the progression of kidney disease (Keane 2000).

Oxidative stress status among the CKD patients was evaluated by measuring plasma lipid peroxidation end product MDA (an important index marker of the extent of lipid peroxidation), whereas antioxidant vitamins (vitamin A and C), uric acid and catalase evaluated the antioxidant status. The increase in MDA found in this study (Table 3.18) is congruent with results of other studies (Fiorillo *et al.*, 1998; Rutkowski *et al.*, 2006) which underpin the fact that MDA is increased in CKD patients compared to controls. This depicts the state of oxidative stress of the CKD patients. Furthermore, levels of MDA increased with the progression of disease from stage 1 to stage 5 which is in accordance with the study of Yilmaz *et al.*, (2006). Lipid peroxidation products may contribute to endothelial injury and may be involved in intensive oxidative modifications of LDL (Esterbauer *et al.*, 1992) and in the development of atherosclerosis (Basha and Sowers, 1996). Peroxidized lipids damage RBCs and other proteins altering their physical properties (Desai and Tappel, 1963).

Moreover, MDA altered LDL-C (Ox LDL) leads to accumulation of cholesterol ester within human monocyte macrophages and it has been hypothesized that modification of native LDL may be a prerequisite for the accumulation of cholesteryl esters within the cells of atherosclerotic reaction (Fogelman *et al.*, 1980). Secondly, increased lipid peroxidation causes endothelial dysfunction through the degeneration of nitric oxide (Taddei *et al.*, 1998). Consequently, the endothelial cells lose their ability to protect the vessel wall and become atherosclerotic promoters (Lüscher and Vanhoutte, 1990). Other mechanisms by which endothelial function is impaired in CKD which were not determined in this study include elevated levels of asymmetric dimethylarginine (ADMA) a competitive inhibitor of endothelial nitric oxide (NO) production (Zoccali *et al.*, 2001) and chronic inflammation (Stenvinkel *et al.*, 1999). Thus lipid peroxidation marked by an increased MDA level may also contribute to the high incidence of premature atherosclerosis in CKD patients.

Catalase is a haemoprotein catalyzing the reduction of hydrogen peroxides and protects against highly reactive hydroxyls. The plasma concentrations of catalase increased significantly in the CKD patients compared with the controls (Table 3.18), and increased with the progression of the condition. This observation is, however, contrary to the findings made in other studies elsewhere (Chen *et al.*, 1997) and may thus require further scientific enquiry.

As one of the most important antioxidants ascorbic acid (vitamin C) is most effeicient at preventing lipid peroxidation and by so doing reduces endothelial dysfunction (Deicher and Horl, 2003). The significant decrease in the plasma concentration of Vitamin C observed in this study (Table 3.18) is consistent with the observations made in other studies (Bakaev *et al.*, 1999). Although in CKD deficiency of vitamin C can be observed, its administration in these patients requires careful consideration. High serum concentrations either from ingested food or as supplementation may lead to hyperoxalaemia that may impact vascular disease in the uraemic patient (Pru *et al.*, 1985).

The elevated plasma retinol (Vitamin A), an antioxidant vitamin, observed among the CKD patients in this study (Table 3.18) is consistent with the findings of Hala *et al.*, (2000). The high level of plasma retinol in CKD may be due to the increased levels of retinol binding protein (RBP), reduced vitamin excretion and decreased conversion of retinol to retinoic acid in the whole blood (Gerlach and Zile, 1990). In renal dysfunction both the excretory and tubular catabolism of RBP are reduced which results in the build-up of these proteins in the blood (Smith and Goodman, 1976). A negative correlation between eGFR and plasma retinol was observed in this study. Ayatse, (1991) and Kaplan *et al.*, (1987) however observed a positive correlation between serum creatinine and plasma retinol.

Uric acid is generated in the human body by the degradation of purines. It has been found that uric acid is a powerful antioxidant and is a scavenger of singlet oxygen and other radicals (Grootveld and Halliwell, 1987). In this study, the significantly higher plasma uric acid concentration that was found compared to the control (Table 3.18), is probably a consequence of the collapse of the excretory function of the kidneys as well as increased protein catabolism as part of the hypercatabolic state in CKD (Davison *et al.*, 1999). The hyperuricaemia of CKD is frequently associated with an increase in the prevalence of gouty arthritis and tophi. It also represents an independent factor for the progression of kidney disease and the risk of cardiovascular disease. High uric acid levels induce vasoconstriction, inflammation and intrarenal angiotensin II production, all of which promote hypertension (Johnson *et al.*, 2005).

4.5 RELATIONSHIP BETWEEN PARATHYROID HORMONE AND ELECTROLYTES IN CKD

This study compared the relationship between serum electrolytes and their ratios with parathyroid hormone in CKD patients. The significant increase in the serum PTH level observed in this report (Table 3.20) among the CKD patients compared

to normal controls, as well as the progressive increase in the serum concentration of PTH as kidney function deteriorates has been observed in earlier studies (Pitts *et al.*, 1988; Levin *et al.*, 2007). Persistent increase in PTH is known to cause secondary hyperparathyroidism. Furthermore, high PTH levels have been implicated in a myriad of abnormalities including cardiovascular, metabolic, haematologic, and immunologic abnormalities (Meytes *et al.*, 1981; Massry and Smogorzewski, 1994; Chiu *et al.*, 2000).

In CKD patients, magnesium is mostly excreted via the renal route; consequently a positive magnesium balance is anticipated (Mountokalakis, 1990) even though normal or decreased values may be found as a result of decreased dietary intake together with diminished absorption as a result of deficient synthesis of the active metabolite of vitamin D by the damaged kidney (Schmulen *et al.*, 1980; Spencer and Osis, 1988). The serum magnesium levels were found to be significantly higher in the cohort of subjects with CKD than in controls, an observation that is consistent with earlier reported findings (Mountokalakis, 1990; Agus and Massry, 1994; Massry and Smogorzewski, 1994). Additionally, the CKD patients were several folds at risk of hypermagnesaemia from this study as compared to the controls.

There have been inconsistent results in the study of the relationship between Mg and PTH in CKD (Massry *et al.*, 1970; Ferment *et al.*, 1987). However, in this study we observed a positive linear relationship between magnesium and PTH (Figure 3.8D), a finding which is consistent with previously reported findings (Wei *et al.*, 2006). Chronic hypermagnesaemia is known to suppress the excretion of PTH in end-stage CKD, which contributes to the calcification of the soft tissues including vascular calcification (Wei *et al.*, 2006). Furthermore, in dialysis patients hypermagnesaemia is known to suppress PTH production, thus it is considered an important factor in the manufacturing of dialysis fluids.

As kidney disease progresses there is diminished filtration and excretion of phosphate resulting in hyperphosphataemia, a finding consistent with observations made in this study. Initially this is surmounted by an elevation in the serum level of PTH which decreases proximal phosphate reabsorption. However, eventually there is hyperplasia and hypertrophy of the parathyroid gland as a result of this physiologic compensation, setting the stage for secondary hyperparathyroidism (SHPT) and a vast array of metabolic, vascular, rheumatologic, and cardiac complications that are associated with its onset (Slatopolsky *et al.*, 1966). The CKD cohorts in this study had significant hyperphosphataemia between stage 3 and 5 which is line with the work of Block *et al.*, (1998) who proved that in the latter stages of CKD the rate of intestinal phosphate absorption exceeds that of urinary phosphate excretion resulting in hyperphosphataemia. The linear relationship observed between PTH and phosphate in this study is in agreement with previously reported works (Slatopolsky and Delmez, 1994; Naveh-Many *et al.*, 1995; Almaden *et al.*, 1998).

Serum potassium is generally believed to rise above the normal limit only at the end stage of CKD (Allon, 1995). This finding is in agreement with observations made in this study. Furthermore, the linear relationship between potassium and PTH observed in this study is in agreement with previously reported findings which explained that excess PTH increases basal levels of cytosolic calcium which affects the permeability of the cellular membrane to potassium thus decreasing extra renal disposal of potassium in CKD (Soliman *et al.*, 1989). Furthermore, potassium is known to stimulate the pancreas to release insulin (Bia and DeFronzo, 1981), an important regulator of extra renal disposition of potassium. Since SHPT of CKD impairs glucose-induced insulin secretion (Fadda *et al.*, 1991), it is likely to interfere with potassium-induced insulin secretion which could result in the derangement of the extra renal disposition of potassium.

The CKD patients in addition to having a significantly lower serum sodium concentration were also about 9 times at risk of developing hyponatraemia as compared to the controls (Table 3.22). This observation is coherent with the findings from earlier studies (Slatopolsky *et al.*, 1968).

Serum calcium levels were found to be significantly lower in the cohorts with CKD than in controls due to impaired intestinal absorption and phosphate retention, a finding that is consistent with previously reported observations (Kurokawa, 1994). Furthermore, the CKD patients were about 8 times at risk of developing hypocalcaemia from this study as compared to the controls. Hypocalcaemia stimulated excess PTH secretion (Yamamoto *et al.*, 1989) as reported in this study.

A lot of attention has been given to the effects of the variations in the inorganic composition of tissue fluids in the diseased kidney, because in the maintenance of health, balance plays an essential role. The understanding of mineral ratios is particularly interesting and more informative than analyzing mineral levels alone. Calcium/Magnesium (Ca/Mg) ratio is otherwise referred to as the blood sugar ratio because whereas calcium is necessary for the discaharge of insulin from the pancreas magnesium inhibits the secretion of insulin. Furthermore, as coregulation ions, magnesium maintains calcium in solution. The study observed a decrease in the Ca/Mg ratio among the CKD subjects coupled with the decrease in the ratio as kidney function deteriorated (Table 3.20 and Table 3.21). An excess amount of magnesium comparative to calcium (low Ca/Mg ratio) will result in decreased calcium mediated insulin secretion (Bennett *et al.*, 1969; Watts, 1986) and hence a high serum glucose level as observed among the CKD patients in this study. The Ca/Mg ratio has been used as an index of vulnerability of urine to form kidney stones in CKD patients (Bastian and Vahlensieck, 1975).

Sodium/Potassium (Na/K) ratio, often referred to as the life-death ratio, is vital because it is linked to the sodium pump mechanism, and the electrical potential of cells which is controlled by sodium and potassium levels. Sodium is normally

extracellular whilst potassium is normally intracellular. A distortion in the ratio of these minerals suggests important physiological malfunction within the cells of CKD patients. Furthermore, the ratio is closely linked to the adrenal gland function and the equilibrium between aldosterone (mineralocorticoid) and cortisone (glucocorticoid) secretion (Watts, 1989). The significantly reduced Na/K ratio observed in the CKD cohort (Table 3.17) may be due to an alteration of the regulatory mechanisms of the renin-angiotensin system.

Otherwise known as the thyroid ratio, the Calcium/Potassium ratio plays an important role in regulating thyroid activity which is associated with the regulation of the metabolic rate in the body (Watts, 1989). Metabolism is faster when the thyroid is hyperactive and the effectiveness of energy production in the body reduces when the thyroid (adrenal) ratios are abnormal. Since the level of Ca/K ratio in the CKD subjects was significantly reduced compared to the controls, it could be inferred that the thyroid function of this cohort is inadequate, a finding harmonious with previously reported studies (Spector *et al.*, 1976).

Sodium/Magnesium ratio (Na/Mg) is also known as the adrenal ratio in tissue reading (hair analysis) due to the direct link sodium has with adrenal gland function. On the contrary this will not match blood tests results for adrenal hormones. Even though variations in adrenal steroids have been noted in CKD patients as observed in this study according to the Na/Mg ratio (Watts, 1989), normal blood tests is a common occurrence, though tissue mineral ratio will show abnormal adrenal function and vice versa for this ratio. Sodium retention in the body is regulated by aldosterone, a mineralocorticoid hormone, with the aldosterone level proportional to the sodium level. The Na/Mg ratio measures the amount of energy produced by the body because the adrenal glands are the foremost regulator of the rate of metabolisbm. The effect of magnesium on adreno-cortical activity leads to increased magnesium retention as reported in previous

studies (Watts, 1989). The sodium-magnesium profile is indicative of reduced adrenal cortical function as observed in this study.

Chapter 5

5.1 CONCLUSIONS

In conclusion, the CKD-EPI, CG and 4v-MDRD are the most accurate equations among the seven predictive equations for renal dysfunction even though they seem to have their limitations. Whereas the sensitivity and the accuracy of the CG formula cannot be overlooked due to the influence of weight, CKD-EPI and the 4v-MDRD equations are also difficult to calculate in clinical practice especially in our setting. However, when CKD-EPI is used bias is enhanced particularly at elevated e GFRs even though precision is not the best. Further studies are therefore, warranted prior to the generalization of these findings for patients presenting with chronic kidney disease.

This study has established that the metabolic syndrome is prevalent in about 30.1% of the study population and that increased WC, BMI, TG and SBP are components of the metabolic syndrome which are independent risk factors for the development of CKD in the Ghanaian population. Furthermore, female subjects with MS are at greater risk of developing CKD compared to their male counterparts. In addition, there is a graded relationship between the number of the metabolic syndrome components and risk of CKD. These findings warrant a clinical evaluation to clarify whether the effect of preventing and/or treating the factors predisposing subjects to the metabolic syndrome will result in improved renal prognosis and substantially ease the burden of CKD in Ghana.

Anaemia is primarily a risk factor for CVD. Furthermore, the presence of anaemia and CKD confers a particularly high-risk group. Although true biologic interaction is rare and the duration of kidney disease, which could explain the observed association, is unknown, the magnitude of the effect may warrant, if these results are confirmed, aggressive prevention strategies of progression of kidney disease and early treatment of anaemia. Clinical trials studying the effect of early treatment of anaemia in patients with kidney disease are necessary, however, before suggesting a change in guidelines.

Dyslipidaemia and increased oxidative stress with abnormal antioxidant levels are common in CKD patients. Based on the findings in this study, it may seem reasonable to propose that therapeutic regimens aimed at strengthening the antioxidant defenses as well as normalizing lipid concentrations would be useful in protecting CKD patients against oxidative stress and any related complications.

The results of the present study suggest that PTH is linked with derangements in the metabolism of electrolytes like calcium, magnesium, phosphorus and potassium in CKD and contributes to a plethora of complications. PTH should be measured early in CKD and the necessary interventions including dietary and pharmaceutical, concerning these electrolytes, provided to protect the CKD patient from any complication that will arise as a result of PTH excess.

5.2 RECOMMENDATIONS

Further studies should be conducted to find the incidence and prevalence of CKD among the general population in Ghana.

The relationship between oxidative stress, CKD and cardiovascular disease needs to be investigated further to establish the role played by the various oxidative stress markers such as nitric oxide.

Furthermore, a cut-off for anaemia in CKD should be established among Ghanaian CKD patients.

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APPENDIX