

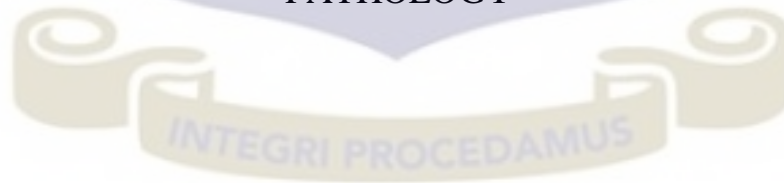
DETERMINATION OF PLASMA LEVELS OF FIBROBLAST GROWTH
FACTOR 23 IN GHANAIAI INDIVIDUALS WITH HYPERTENSION AND
CHRONIC KIDNEY DISEASE

BY

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THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON
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PATHOLOGY



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DECLARATION

I, BIEKPE SYLVESTER, do hereby declare that, with the exception of references to people's work, which have been duly acknowledged, this thesis is the outcome of my own research conducted at the Department of Chemical Pathology, University of Ghana, College of Health Sciences under the supervision of Prof. Henry Asare-Anane and Dr. Charlotte Osafo. No part of this work has been presented elsewhere for the award of any degree or purpose.

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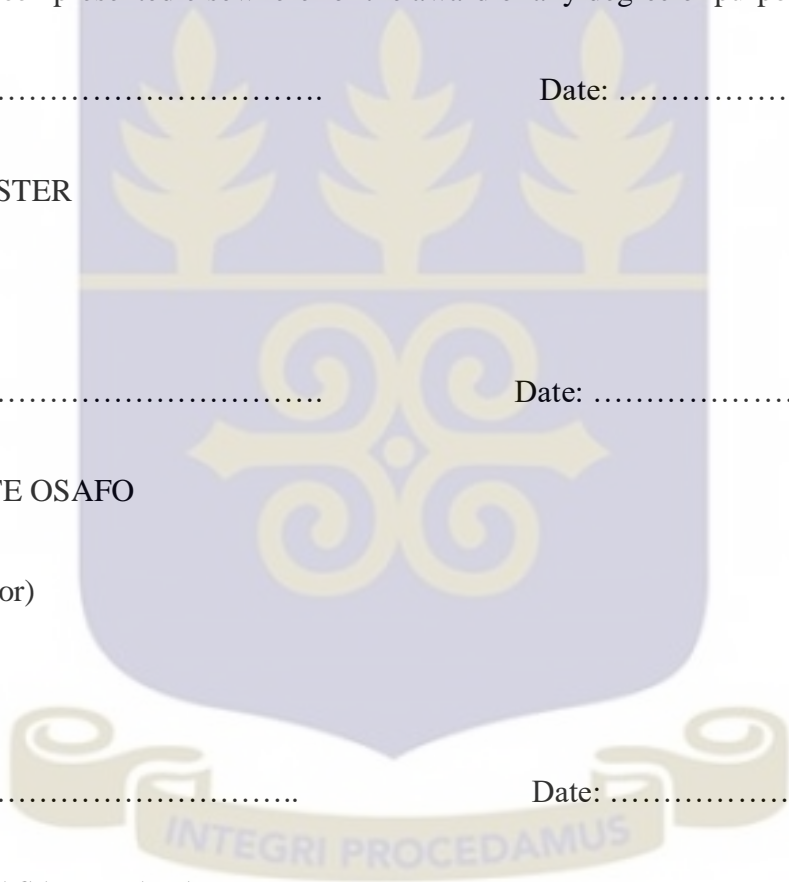
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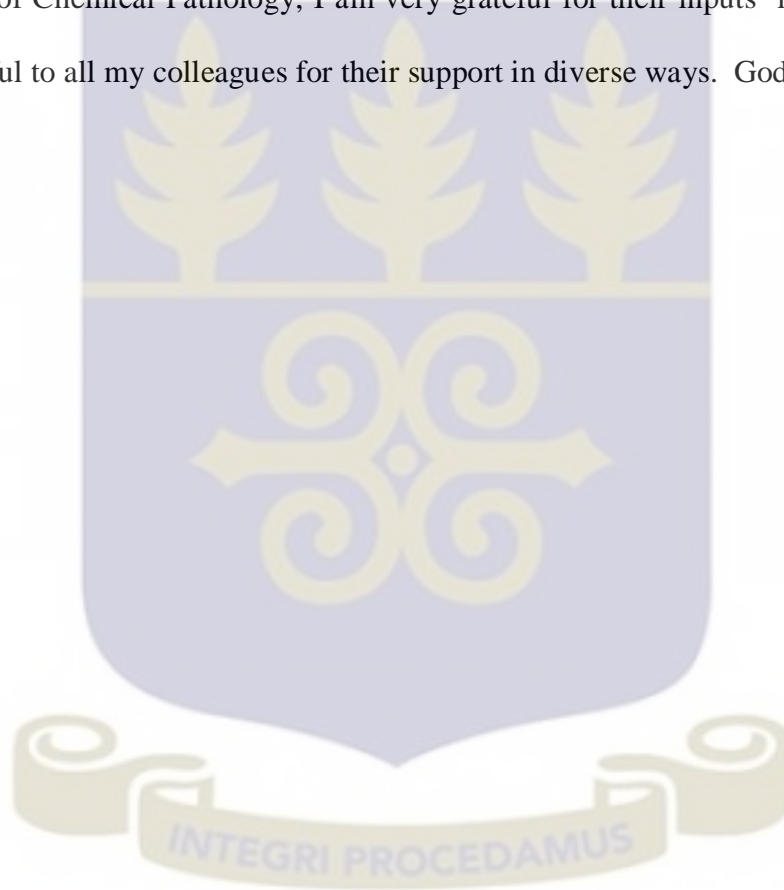
DEDICATION

I dedicate this work to Almighty God, the Biekpe Family of Duong and my future wife.



ACKNOWLEDGEMENT

I duly acknowledge the insightful contributions by my supervisors, Prof. Henry Asare – Anane, and Dr. Charlotte Osafo whose criticisms, suggestions and corrections have helped me to produce this work. My sincere thanks also go to my Prof. Robert Yennah, Hon. Roger Angsowmine, and Langu T.K Andrews for their support and to all the hardworking staff in the Department of Chemical Pathology, I am very grateful for their inputs in various ways . I am equally grateful to all my colleagues for their support in diverse ways. God blesses them all.



ABSTRACT

Background: Fibroblast growth factor 23 (FGF23) is a newly found hormone that promotes renal phosphate excretion. It is secreted by osteocytes. In progressive chronic kidney disease (CKD), FGF23 levels increase in parallel with the deterioration of renal function, thus serves as a novel risk marker for progression of chronic kidney disease. The physiologic stimulus for FGF23 secretion is linked to hyperphosphatemia caused by phosphate load. Elevated FGF23 levels have spurious effects such as vascular calcification, left ventricular hypertrophy, end stage renal disease and death especially in renal impaired patients and patients at risk of developing renal impairment such as hypertensive patients.

Aim: The aim of the study was to measure the levels of FGF23 in non-hypertensive, hypertensive and chronic kidney disease patients.

Specific objectives: The study determined and compared the level of FGF23 among normotensive, hypertensive, and CKD individuals as well as identified risk factors associated with increase in FGF23 plasma level in individuals with hypertension.

Materials and methods: The study was a hospital based case control study in which purposive sampling was used to select the study subjects. It was conducted at the Holy Family Hospital, Techiman. In all, 146 respondents took part in the study. A structured questionnaire was used to gather their anthropometric and demographic characteristics. Blood and urine samples were taken for biochemical analysis for urea, creatinine, sodium, calcium, phosphate, and albumin whiles FGF23 was analyzed from citrated plasma.

Results: There were 86 study subjects with hypertension and 60 without hypertension. Out of the 86 subjects with hypertension, 24 (27.9%) had CKD, ranging from stages 2 to 4. There was significant difference in the plasma levels of FGF23 among the subjects with hypertension, CKD and those without hypertension ($P < 0.05$). Again, there was significant difference in FGF23 levels in all the stages of CKD. A significant inverse correlation was found between FGF23 levels and estimated GFR ($r = -0.505$, $P = 0.000$), calcium ($r = -0.568$, $P = 0.000$) in both hypertensive and CKD subjects. A significant direct correlation between FGF23 levels and urea ($r = 0.700$, $P = 0.000$), creatinine ($r = 0.715$, $P = 0.000$) and phosphate ($r = 0.679$, $P = 0.000$) was reported. Systolic and diastolic blood pressure also correlated significantly and directly with FGF23 levels in subjects with hypertension ($P = 0.000$) but not statistically significant in those with CKD ($P > 0.05$). Hypertensive individuals on angiotensin converting enzyme inhibitors ($OR = 9.1 \times 10^7$, 95% CI: $1.1 \times 10^6 - 6.9 \times 10^7$, $P < 0.0001$) were at risk of increasing their FGF23 plasma levels. Systolic and diastolic blood pressure, urea, creatinine and phosphate were all significant risk factors for increasing plasma FGF23 levels ($OR > 1$, $P < 0.05$).

Conclusion: There is significant difference in the plasma levels of FGF23 between individuals with hypertension and CKD. Angiotensin converting enzyme inhibitors, phosphate load, urea, and creatinine concentrations were risk factors for increasing the plasma levels of FGF23 in hypertensive patients.

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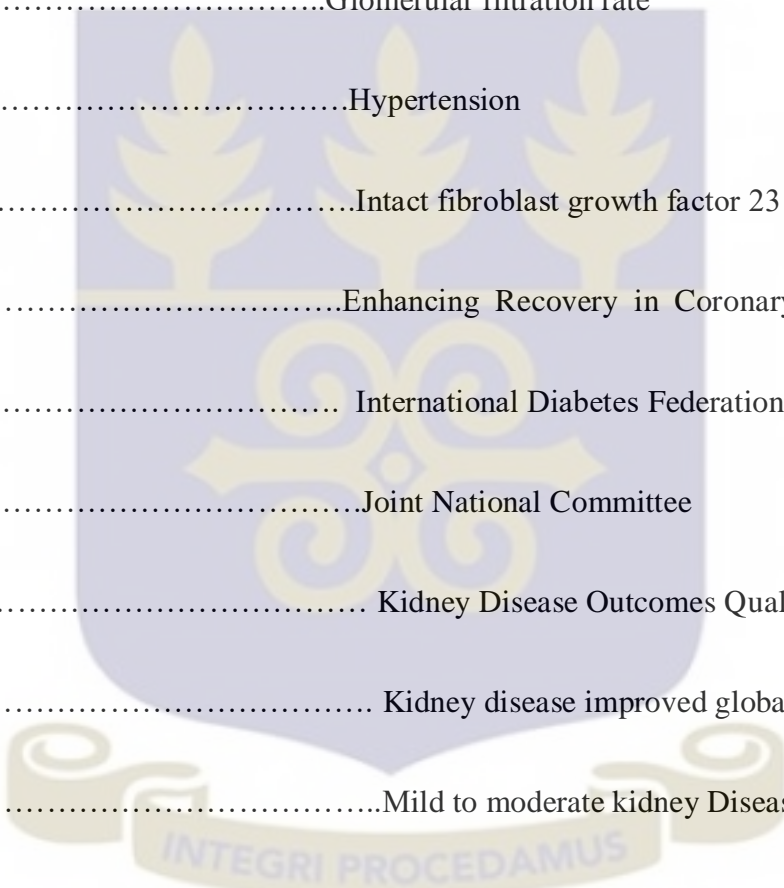
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ABBREVIATIONS

ADHR.....	Autosomal dominant hypophosphatemic rickets
ACE	Angiotensin Converting Enzyme
ACEI.....	Angiotensin converting enzyme inhibitors
CaCB.....	Calcium channel blockers
AusDiab	Australian Diabetes, Obesity and Lifestyle
ANOVA.....	Analysis of Variance
(BUN).....	Blood urea nitrogen
(BMI)	Body mass index
cFGF23.....	C-terminal fibroblast growth factor 23
CRIC.....	Chronic Renal Insufficiency Cohort
CKD	Chronic kidney disease
CKD-EPI.....	Chronic Kidney Disease Epidemiology Collaboration
CI	Confidence interval
DBP	Diastolic blood pressure
GFR	Estimate glomerular filtration rate
ELISA	Enzyme-linked immunosorbent assays



ESRD.....	End stage renal disease
FGF23.....	Fibroblast growth factor 23
FGFRs.....	Fibroblast Growth Factor receptors
GNA.....	Ghana news agency
GFR.....	Glomerular filtration rate
HTP.....	Hypertension
iFGF23.....	Intact fibroblast growth factor 23
ENRICHD	Enhancing Recovery in Coronary Heart Disease
IDF.....	International Diabetes Federation
JNC	Joint National Committee
K/DOQI	Kidney Disease Outcomes Quality Initiative
(K/DIGO)	Kidney disease improved global outcomes
MMKD	Mild to moderate kidney Disease
MDRD.....	Modification of Diet in Renal Disease
nFGF23.....	N-terminal fragment
NIH	National Institute of Health
<i>NHANES III</i>	Third National Health and Nutrition Examination Survey

NKF.....National Kidney Foundation

NTP.....Normotensive

OROdd ratio

PTH..... parathyroid hormone

PUR.....Plasma urea

PCR.....Plasma creatinine

RAS..... Renin Angiotensin System

RXXXarginine – X – X – arginine

NPT..... sodium-dependent phosphate co-transporters

SP..... signal peptide

SEM.....Standard error mean

SPSS.....Statistical package for social scientist

SBP.....Systolic blood pressure

USA.....United States of America

USRD.....United States renal data

UACR..... urine albumin-creatinine ratio

VISP..... Vitamin Intervention in Stroke Prevention

1,25 (OH)₂D₃ 1,25-dihydroxyvitamin D

%.....percentage



CHAPTER ONE

1.0 INTRODUCTION

1.1 Study Background

Fibroblast growth factor 23 (FGF23) is identified to be a phosphate-regulating hormone that is produced primarily by osteocytes (Riminucci *et al.*, 2003; Pereira *et al.*, 2009; Liu *et al.*, 2006). FGF23 expression is predominantly regulated by plasma phosphate (Gutierrez *et al.*, 2009) and 1, 25-dihydroxyvitamin D (Nishi *et al.*, 2005). The principal target organ of FGF23 is the kidney where it causes the internalization of sodium – phosphate co transporters in renal tubular cells and the suppression of 1 α -hydroxylase activity (Juppner *et al.*, 2010) thus decreasing plasma phosphate by increasing urinary phosphate excretion and down-regulating 1,25-dihydroxyvitamin D concentrations. Again, FGF23 inhibits parathyroid hormone (PTH) secretion (Ben-Dov *et al.*, 2007). It is elevated in most conditions that are associated with hyperphosphatemia; renal failure, severe catabolic states and vitamin D toxicity (Fukagawa and Kazama, 2005). The effects of FGF23 in the kidney and parathyroid glands are mediated when it binds to FGF receptors (FGFRs) complexed to the co-receptor *Klotho* (Urakawa *et al.*, 2006).

Fibroblast Growth Factor-23 is secreted in response to 1,25 (OH)₂D₃ load which increases the mRNA levels that encodes it (Kolek *et al.*, 2005). The action of FGF23 is mediated in the kidney through an FGF receptor (FGFR)/*Klotho* complex to down-regulate NPT2a and NPT2c expression in the proximal tubules. In chronic kidney disease (CKD) patients Fibroblast growth factor 23 plays a beneficial role by preventing phosphate toxicity (Isakova *et al.*, 2011). This helps in maintaining phosphate balance in the patients as it stimulates renal phosphate excretion. Serum levels of FGF23 increase gradually as kidney function deteriorates. In the early and

intermediate stages of CKD, FGF23 levels are increased often to 2–5 times the upper limit and can reach more than 200 times in cases of advanced renal failure in an attempt to mediate phosphate balance (Gutierrez *et al.*, 2009).

Although FGF23 helps to prevent hyperphosphatemia, elevated circulating levels are thought to exert a negative effect on the CKD patient. It is reported that elevated level of FGF23 are independently associated with vascular dysfunction (Yilmaz *et al.*, 2010), left ventricular hypertrophy (Gutierrez *et al.*, 2005), increased risk for end stage renal disease (ESRD) and death in patients with CKD (Isakova *et al.*, 2011). Isakova *et al.* (2015) however, has reported that higher fibroblast growth factor 23 levels are not independently associated with higher risk of incident CKD in patients with type 2 diabetes. Recent data indicate that high levels of FGF23 contributes directly to the development of left ventricular hypertrophy, suggesting a possible biologic basis for the observed association between elevated FGF23 and mortality (Faul *et al.*, 2011). According to Herald (2011), in late CKD, FGF23 cannot reduce serum phosphate levels, and abnormally high plasma FGF23 concentrations appear to exert spurious off-target effects.

The FGF 23 gene encodes the 251 amino acid FGF 23 peptide, which includes a signal peptide (SP) of 24 amino acids. Prior to secretion, the SP is cleaved to form the intact FGF23 protein. The intact FGF23 protein contains the arginine–arginine (RXXR) motif which is a protease recognition site (Benet-Pages *et al.*, 2004). When proteolytically cleaved between Arg-179 and Ser-180, the intact FGF23 (~32 kDa) forms an N- and C- terminal (~12 kDa) fragment. It is thought that only the intact FGF23 protein is biologically functional and that the cleavage step forming the N- and C-terminal fragments renders the protein inactive (Shimada *et al.*, 2001). The amino-terminal portion of FGF-23 (1-24) is hydrophobic and serves as a signal peptide allowing its secretion into the blood circulation. Its carboxyl-terminal portion (180-

251) shares only limited amino acid homology with other members of the FGF family of proteins. Since the level FGF23 rises in early CKD (Gutierrez *et al.*, 2005), it may be used to indicate the onset of clinically detectable reductions in eGFR in which FGF23 could serve as a screening test for CKD in hypertensive patients, with high levels acting as either a biomarker of subclinical kidney disease yet to be reflected in a reduced eGFR.

1.2 Problem Statement

The challenge with the diagnosis and management of hypertension and its association with CKD is the unavailability of diagnostic markers (Rudijanto, 2006). Blood pressure measurement is commonly relied upon for diagnosing and managing hypertension. However, errors in blood pressure measurements due to several factors such as human error, auscultatory gap caused by venous distension, and patients with prosthetic aortic valves or arteriosclerosis make the use of blood pressure measurement for diagnosing and managing hypertension a dilemma (Rudijanto, 2006). Thus, hypertensive patients continue to suffer premature death from cardiovascular diseases and CKD. The progression of CKD, development of cardiovascular diseases, and premature death has been linked to high plasma levels of FGF23. Studies have shown that FGF23 contribute to the development of hypertension through sodium retention and CKD (Olena *et al.*, 2013), thus providing a link between hypertension and CKD. However, the plasma level of FGF23 and its association with blood pressure has not been examined. The level of FGF23 in the various stages of hypertension has also not been well elucidated. Also, FGF23 is elevated in the early stages of CKD and predicts outcomes in CKD (Gutierrez *et al.*, 2009). Studies have shown that abnormally high level of FGF23 is independently associated with CKD mortalities (Isakova *et al.*, 2011), left ventricular hypertrophy (Gutierrez *et al.*, 2009), vascular dysfunction and calcification (Yilmaz *et al.*, 2010; Olena *et al.*, 2014), uncontrolled hypertension and

cardiovascular diseases (Olena *et al.*, 2013). Similarly, high FGF23 concentrations have been found to be associated with the progression of CKD to end stage renal disease (Danilo *et al.*, 2007). Despite the linkage of FGF23 to structural or dynamic cardiovascular changes such as stroke, vascular dysfunction, and left ventricular hypertrophy, the plasma level of FGF23 in individuals with hypertension is unassessed. Again the association of CKD markers (urea, creatinine, urine-albumin creatinine ratio and Glomerular Filtration Rate) and FGF23 has not been well elucidated in people with hypertension. Furthermore, antihypertensive medications and blood pressure as risk factors for increasing FGF23 remains elusive. Therefore, assessing the plasma level of FGF23 will likely serve as a tool for monitoring the progress of hypertension and hypertension to CKD. This study therefore seeks to fill these gaps and determine the plasma level of FGF23 in hypertensive and CKD patients and provide data on the level of FGF23 to guide the management of hypertensive patients in order to avert the negative effects of high FGF23.

1.3 Justification

In Sub-Saharan Africa, hypertension is one of the leading causes of CKD (Naicker, 2013). The prevalence of hypertension in Ghana is 25.4% (Addo *et al.*, 2012) and a 46.9% prevalence of CKD in outpatient hypertension in Ghana (Osafo *et al.*, 2011). Early detection and good management of hypertension is the surest way of preventing renal damage. Controlling the level of FGF23 could be a possible way of controlling high blood pressure and prevention of hypertensive nephropathy, vascular calcification and CKD progression. Since increased levels of FGF23 is detectable in the early stages of CKD its level could be used in association with blood pressure as a screening test of hypertensive nephropathy and to monitor the progress of hypertension. There is therefore the need for a comprehensive approach for the inclusion of

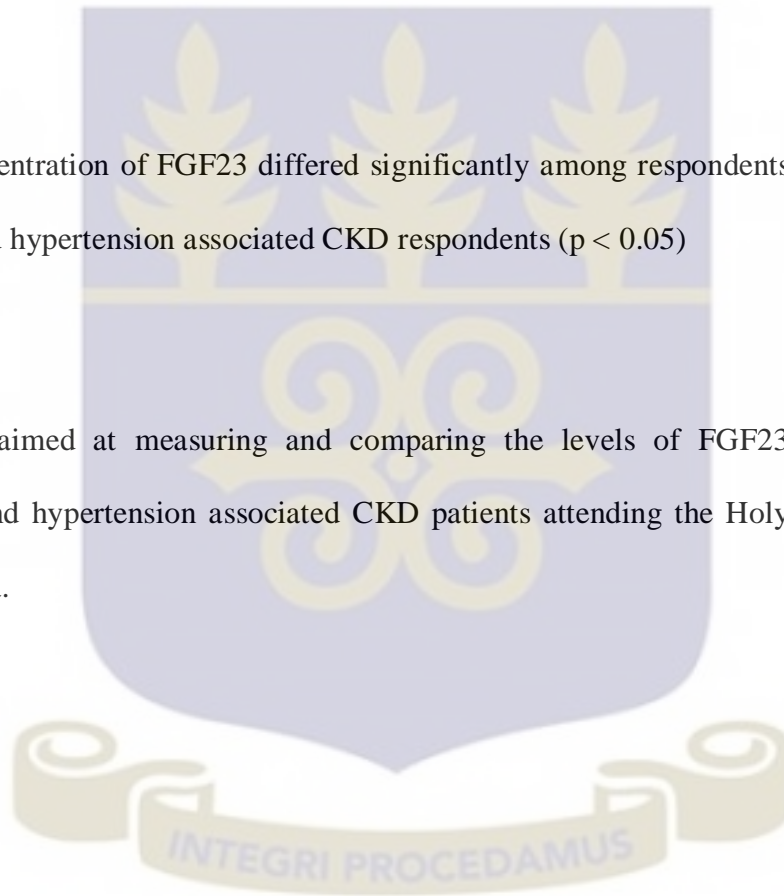
FGF23 in the management and prevention of hypertension. It is anticipated that this study will provide relevant information that could be used to guide strategies for the management of phosphate and other mineral imbalance in patients with hypertension and renal patients in order to improve clinical outcomes. Furthermore, the level of FGF23, if elevated, could make FGF23 a useful biomarker or screening test of hypertension among risk groups and serve as a therapeutic target to avert the reported negative effects of elevated circulating FGF23.

1.4 Hypothesis

The plasma concentration of FGF23 differed significantly among respondents with and without hypertension, and hypertension associated CKD respondents ($p < 0.05$)

1.5 Aim

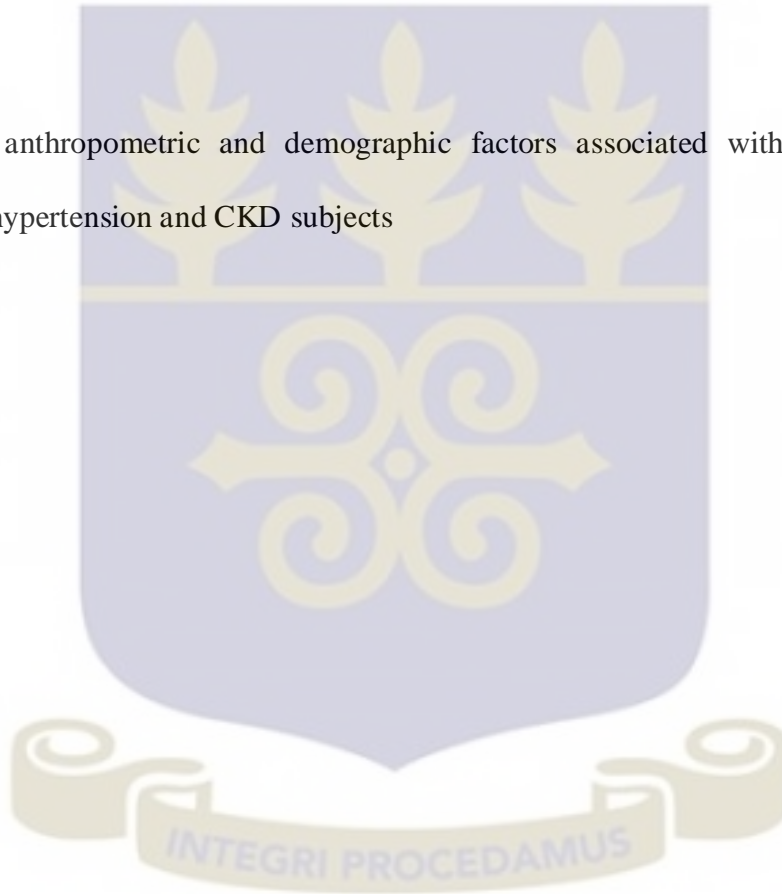
The study was aimed at measuring and comparing the levels of FGF23 in hypertensive, normotensives and hypertension associated CKD patients attending the Holy Family Hospital, Techiman, Ghana.



1.6 Specific Objectives

The objectives of the study were to:

- i. Determine and compare the plasma concentration of FGF23 among hypertensive, normotensive and hypertension associated CKD subjects.
- ii. Determine the correlation between FGF23 and demographic, anthropometric and biochemical characteristics.
- iii. Identify the anthropometric and demographic factors associated with elevated FGF23 concentration in hypertension and CKD subjects



CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Chronic Kidney Disease

Chronic kidney disease (CKD) is a public health issue that affects millions of people in both developing and developed countries. The presence of CKD predisposes individuals to high risks of end-stage renal disease, cardiovascular disease and premature death. CKD is one of the most potent known risk factors for cardiovascular disease, such that individuals with CKD have a 2 to 3-fold greater risk of cardiac death than age- and sex-matched controls without CKD (Weiner *et al.*, 2004). Chronic Kidney Disease directly or indirectly contributes to the deaths of approximately 10% of Australians and is one of the few diseases in which mortality rates are worsening over time (Li *et al.*, 2004). However, timely identification and treatment of CKD can decrease the risks of cardiovascular disease and CKD progression by up to 50% (Johnson, 2004). The Kidney Disease Outcomes Quality Initiative (K/DOQI 2002) of the National Kidney Foundation (NKF) developed a practice guideline for CKD which was later revised and improved by the kidney disease improved global outcomes (K/DIGO, 2012). According to both guidelines, CKD is defined as either kidney damage or glomerular filtration rate (GFR) below 60 ml/min/1.73 m² for at least three months with or without evidence of kidney damage, irrespective of the cause when GFR is estimated by serum creatinine based on equations rather than on direct measurements. In the USA, the third National Health and Nutrition Examination Survey (*NHANES III*) estimated that the prevalence of CKD has recently risen from 11% between 1988 and 1994 up to 13% between 1999 and 2004. In England, a

prevalence of 6.76% was recorded compared to a national prevalence of 4.3% (Benjamin *et al.*, 2013).

Studies have shown a prevalence of CKD of 10.4% in Nigeria (Afolabi *et al.*, 2009) and 36% in Congo (Sumaili *et al.*, 2009). Not less than 27 million Nigerians have chronic kidney disease with incidence of 100 per million population (i.e., 15,000 new cases every year) and prevalence of 300 to 400 million (that is 45,000 living with kidney failure annually), according to the National Kidney Foundation (NKF, 2012). The incidence of CKD in Nigeria has been shown by various studies to range between 1.6% and 12.4% (Odubanjo *et al.*, 2011). In the Democratic Republic of Congo, Kinshasa, the prevalence of all stages of CKD according to K/DOQI guidelines was 12.4% (Sumaili *et al.*, 2008). In Ghana, the prevalence of CKD in hypertensive patients is 46.9% (Osafo *et al.*, 2011). Tanzania has an alarming CKD population; 83.7% had CKD, 24.7% had eGFR < 60 mL/min, 80.0% had moderately to severely increased albuminuria, 34.1% had overt proteinuria and 45.8% had microalbuminuria alone in a study conducted among adult diabetic population (Janmohamed *et al.*, 2013). The USRD Data Report (2005) showed that 45% and 27% of diabetes and hypertension respectively caused kidney disease. In Ghana, kidney patients as young as 16 years have been reported at the Korle Bu Teaching Hospital (GNA, 2012) and that most of them fall between 20 and 50 years of age, indicating that kidney diseases in the country are increasing at an alarming rate irrespective of age. In 2009, 387 (16%) of the 2,332 admissions at the unit of the in-patients were kidney related. In 2012, the number of kidney cases increased to 437 (21.4%) out of 2,491 admission which again increased to 627 (23.3%) in 2011 according to available data at the renal unit of the hospital.

2.2 Risk Factors for Chronic Kidney Disease

Several studies have identified various risk factors to be associated with the development of CKD and which can be remedied or potentially modified, in order to detect early CKD and intervene at the earliest possible stage.

2.2.1 Obesity and Chronic Kidney Disease

Obesity is defined as a body mass index (BMI) greater than 30 kg/m². It has become an important public health challenge worldwide especially developed countries. In Australia, the prevalence of obesity in the Australian adult population is 20.5%, which is more than double the prevalent rate observed in 1980 (Dunstan *et al.*, 2000). Similar trends have been observed in the United States of America (Chen *et al.*, 2004). The obesity pandemic appears to be driving secondary epidemics of type II diabetes mellitus and hypertension, which in turn have led to rising rates of patients with CKD (McCullough, 2004).

In earlier studies, Weisinger *et al* (1974) reported an association between obesity and nephrotic proteinuria. The development of glomerulomegaly and focal segmental glomerulosclerosis has since been linked to massive obesity (Jennette, 1987; Kasiske and Crosson, 1986; Kasiske and Napier, 1985; Warnke and Kempson, 1978). Unfortunately, all of these associations have been limited to case reports or small autopsy series. A review of renal biopsies from 1986 to 2000 by the Renal Pathology Laboratory of Columbia Presbyterian Medical Center revealed a progressive increase in biopsy incidence of obesity-related glomerulopathy ranging (Kambham *et al.*, 2001) from 0.2% in 1986-1990 to 2.0% in 1996-2000.

2.2.2 Diabetes Mellitus and Chronic Kidney Disease

Available data suggest that approximately one-third (25-45%) of patients with diabetes mellitus developed CKD (Newman *et al.*, 2005; Parving *et al.*, 1988). Approximately 20%-30% developed microalbuminuria by 15 years, of which less than half progressed to overt nephropathy whilst the remainder were either stable or regressed (Newman *et al.*, 2005). Observational cohort studies suggest that the risk of developing CKD is comparable in both type 1 and type 2 diabetes mellitus and that the risk is primarily determined by glycaemic control, as determined by glycated hemoglobin (Newman *et al.*, 2005; UKPDS, 1998).

2.2.3 Smoking and Chronic Kidney Disease

Numerous studies have suggested that smoking is associated with renal failure progression in both diabetic and non-diabetic CKD (Almdal *et al.*, 1994; Gambaro *et al.*, 2001; Rossing *et al.*, 2002). In a case-control analysis of non-diabetic participants of the Cardiovascular Health Study Cohort, aged ≥ 65 years, the adjusted odds ratio for serum creatinine increased linearly with cigarette consumption to almost 5-fold (Bleyer *et al.*, 2000). However, it is important to note that only 2.8% of the population experienced an increase in serum creatinine, and only 8.8% of men and 9.8% of women in the study were current smokers, resulting in an increase in serum creatinine of 0.3 mg/dL in only 14 smokers according to the researchers. Other cohort studies suggest that cessation of smoking may reduce renal failure progression in diabetic and non-diabetic CKD (Sawicki *et al.*, 1994; Schiffli *et al.*, 2002; Chase *et al.*, 1991).

2.2.4 Alcohol and Chronic Kidney Disease

Researchers have shown that chronic alcohol intake is linked with hypertension and therefore indirectly with CKD (Corrao *et al.*, 2000; Parekh and Klag, 2001). However, there is contrasting epidemiological evidence with some studies demonstrating that moderate-to-heavy alcohol consumption is an independent risk factor for CKD (Perneger *et al.*, 1999; Shankar *et al.*, 2006; White *et al.*, 2009), some studies suggesting no association between alcohol intake and CKD risk (Buja *et al.*, 2011; Menon *et al.*, 2010) while other studies demonstrated an inverse association between alcohol intake and CKD risk (Reynolds *et al.*, 2008; Schaeffner *et al.*, 2005). In the AusDiab study (White *et al.*, 2009), alcohol intake of ≥ 30 g/day was associated with an increased risk of albuminuria after adjustment for age, sex and baseline kidney function, but a reduced risk of eGFR < 60 mL/min/1.73 compared with consumption of < 10 g/day.

2.2.5 Cardiovascular Disease and Chronic Kidney Disease

Several studies have reported that CKD is associated with a greatly increased risk of cardiovascular disease (Foley *et al.*, 1998; Keith *et al.*, 2004; Weiner *et al.*, 2004). In both Enhancing Recovery in Coronary Heart Disease I (ENRICHD), and Vitamin Intervention in Stroke Prevention (VISP) trial studies, the prevalence of CKD (27-28%) in both studies was much higher than in the general population (Bang *et al.*, 2009). Moreover, the presence of cardiovascular disease is independently associated with kidney function decline and with the development of kidney disease. Elsayed *et al.* (2007) reported in their study that baseline cardiovascular disease was associated with an increased risks of serum creatinine elevation, eGFR decline, development of CKD. Based on these studies and others, recently published

guidelines from a joint science advisory committee from the American Heart Association Kidney and Cardiovascular Disease Council; the Councils on High Blood Pressure Research, Cardiovascular Disease in the Young, and Epidemiology and Prevention; and the Quality of Care and Outcomes Research Interdisciplinary Working Group recommended that all patients with CVD should be screened for CKD (Brosius *et al.*, 2006).

2.2.6 Ageing and Chronic Kidney Disease

There is ongoing debate as to whether this age-related GFR decline is normal or pathological. After the age of 30 years, GFR declines progressively at an average rate of 8 mL/min/1.73 m² per decade (Coresh *et al.*, 2003). Based on North American data, Coresh and colleagues (2003) estimated that 25% of the Australian population over the age of 70 years will have an eGFR below 60 mL/min/1.73m² while a recent analysis of the Australian Diabetes, Obesity and Lifestyle (AusDiab) study suggested that over one-third of patients over the age of 65 years had a GFR between 45 and 60 mL/min/1.73 m² (White *et al.*, 2009) though such population studies have been significantly limited by the fact that only single measurements of serum creatinine and urinary markers of kidney damage were performed, thereby likely to overestimate the true prevalence of CKD. Other observational cohort studies suggest that age-related decline can be largely attributed to co-morbidities, such as heart failure (Danilo *et al.*, 1997) and co-existing cardiovascular disease (Baggio *et al.*, 2005). Again, the researchers reported that an eGFR <45 mL/min/1.73 m² predicts significantly increased risks of cardiovascular disease and CKD progression in all age groups and should therefore generally be considered pathological (i.e. CKD) rather than physiological or age-appropriate. An eGFR between 45 and 60 mL/min/1.73 m² is predictive of significantly increased risks of adverse clinical outcomes in younger patients (<65-

70 years), although the benefits of identifying older people with an eGFR >45 mL/min/1.73 m² (Roderick *et al.*, 2009). Based on this evidence, the Australasian Creatinine Consensus Working Group concluded that it was premature to recommend age-related decision points for eGFR but that it was appropriate to advise practitioners that in those patients of 70 years and older with an eGFR from 45 to 59 mL/min/1.73m², when stable over time and unaccompanied by other evidence of kidney damage, the GFR value may be interpreted as consistent with a typical eGFR for this age and unlikely to be associated with CKD complications (Mathew *et al.*, 2007) but for patients younger than 70 years, an eGFR <60 mL/min/1.73m² for at least 3 months is considered diagnostic of CKD.

2.2.7 Hypertension

Hypertension is a condition of sustained systolic/diastolic blood pressure greater than 140/90mmHg (Osafo *et al.*, 2011). It is associated with an increased risk of other diseases. Hypertension can result from an increase in peripheral resistance or cardiac output, or both. The main abnormality in most cases of hypertension is increased total peripheral resistance (Rang and Dale, 2003). In the majority of patients the cause of hypertension is not known, with 95% of all hypertensive patients classified as having primary (essential) hypertension (WebMD, 2012). According to Naicker (2003), hypertension is a leading cause of CKD in Sub-Sahara Africa, ranging from 25% in Senegal to 29.8% in Nigeria, 45.6% in South Africa, and 48.7% in Ghana. It is observed that hypertension affects about 25% of the adult population and is the main cause of ESRD in 21% of patients on renal transplant in South Africa as recently indicated by the South African Renal Transplant Registry. The clinical pattern of hypertension in hospitalized patients takes a rapid course with uremia and death, frequently from cerebral hemorrhage. In Ghana, out of 365 outpatients with hypertension, 110 (30.2%) had serum creatinine >140 µmol/L

(1.6 mg/dL), 48 had serum creatinine >400 $\mu\text{mol/L}$ (>4.5 mg/dL), and 96 (25.5%) had proteinuria (Osafo *et al.*, 2011). The prevalence of hypertension in adults in Ghana is over 28% (Amoah, 2003; Cappuccio *et al.*, 2004), higher in urban than rural areas in Ghana, ranging from 19.3% in rural to 54.6% in urban areas (Addo *et al.*, 2012). The levels of hypertension detection, treatment and control in Ghana are generally low which ranged from 1.7% to 12.7% (Addo *et al.*, 2012). Hypertension has long been documented as a cause, consequence and progressor of CKD. Data from several renal databases identifies systemic hypertension as the second most common cause of ESRD, with Diabetes mellitus being the first. In the United States (US), hypertension is the leading cause of ESRD in African-American patients (USRDS 2010). A study in Washington County reported that the adjusted ratio (95% confidence interval) of developing CKD among women was 2.5 (0.05 to 12.0) for normal blood pressure (BP), 3.0 (0.6 to 14.4) for high-normal BP, 3.8 (0.8 to 17.2) for stage 1 hypertension, 6.3 (1.3 to 29.0) for stage 2 hypertension, and 8.8 (1.8 to 43.0) for stages 3 or 4 hypertension compared with individuals with optimal blood pressure (Haroun *et al.*, 2003). In men, the relationship was similar but weaker than in women. Again, the Framingham Offspring Cohort study reported that systolic blood pressure was a significant independent risk factor for the development of new-onset stage 3 CKD (Foster *et al.*, 2008). In evaluating the development of end-stage renal disease (stage 5 CKD) in men aged 35 to 57 years, who were screened between 1973 and 1975 for entry into the Multiple Risk Factor Intervention Trial and followed until 1990, a strong, graded relation between both systolic and diastolic blood pressure and end-stage renal disease was identified, independent of associations between CKD and age, race, income, use of medication for diabetes mellitus, history of myocardial infarction, serum cholesterol concentration, and cigarette smoking (Klag *et al.*, 1996). Klag and colleagues

(1996) indicated that the adjusted relative risk increased from 1.0 in those with optimal blood pressure (<120/80) to 1.9 with high normal blood pressure, 3.1 with mild hypertension, 6.0 with moderate hypertension, and 11.2 with severe hypertension. An association between blood pressure and the risk of developing CKD has also been reported in other longitudinal studies (Haroun *et al.*, 2003), in cross-sectional studies in Norway (Hallan *et al.*, 2006), in USA (Coresh *et al.*, 2003) and in Australia (Chadban *et al.*, 2003). The degree of renal protection afforded by blood pressure reduction appears to be proportional to the degree of baseline proteinuria and its reduction following treatment (Gisen, 1997; Klahr *et al.*, 1994; Ruggenti *et al.*, 1998).

2.2.7.1 Classification of hypertension

Classification of hypertension is necessary because it provides definitions as to when hypertension begins and helps to assess risk, determines prognosis, and ascertains when and how aggressively to treat. If clinicians are better able to select individuals at high risk, their therapeutic efforts to prevent complications are much more likely to be effective and spare those at low risk from the potential harm of unnecessary or overaggressive therapy. Systems in current use rely on either the level of blood pressure (diastolic, systolic, or both) and classify patients based on the level of relative risk, absolute risk, or both. Based on the epidemiologic study on lifetime risk of hypertension and the impressive increase in the risk of cardiovascular complications associated with levels of blood pressure previously considered normal, the Joint National Committee (JNC) introduced blood pressure classification. Whereas the JNC-6 has classified blood pressure into three stages (1-3), JNC-7 classified blood pressure into two stages, combining stages 2 and 3 of the JNC-6 (Chobanian *et al.*, 2003). Both JNC-6 and JNC-7 however classified a blood pressure greater than or equals 140/90 as hypertension.

Table 2.1. Blood pressure classification (JNC VI, 1997)

<i>Systolic/Diastolic Pressure</i>	<i>Blood</i>	<i>JNC-6</i>	<i>JNC-7</i>
$\geq 140/90$		Hypertension	Hypertension
140/90 – 159/99		stage 1	stage 1
160/100 – 179/109		stage 2	stage 2
$\geq 180/110$		stage 3	stage 2

2.2.7.2 Hypertension and Chronic Kidney Disease

Researchers have shown that there is a strong relationship between hypertension and Chronic Kidney Disease CKD (Roderich *et al.*, 2009; USRDS, 2010). Whereas hypertension is an identifiable cause of End-Stage Renal Disease (ESRD), contributing to the disease itself and its progression, hypertension is also highly prevalent in CKD patients, playing a role in the high cardiovascular morbidity and mortality Ong (2007). The prevalence of hypertension-associated CKD and the burden of cardiovascular morbidity and mortality have been dramatically increasing worldwide (Ong *et al.*, 2007).

The exact mechanism of kidney damage in patients with hypertension is still hypothetical. However, two complementary pathogenic mechanisms that result in kidney fibrosis and scarring have been proposed by Morgado and Pedro (2012). According to them, changes in systemic and renal macro and microvasculature lead to the loss of renal auto-regulation with elevation of intraglomerular capillary pressure and consequent hyperfiltration mediated injury. Hyperfiltration leads to loss of proteins which promotes the release of cytokines and growth

factors by mesangial cells and downstream tubular epithelial cells. Again, Morgano and Pedro (2012) proposed that endothelial dysfunction and loss of endogenous vasodilators are precipitating factors of hypoxic-ischemic injury. The consequent activation of the intrarenal Renin Angiotensin System (RAS) and the increased release of cytokines and growth factors with recruitment of inflammatory cells stimulate apoptosis causing loss of normal kidney cells and increased matrix production, finally leading to progressive glomerular and interstitial fibrosis and scarring. Increased glomerular perfusion and elevation of glomerular capillary pressure resulting in hyperfiltration result in further damage of the affected glomerulus and to increased filtration of proteins to the tubular lumen (Klahr *et al.*, 1988). Furthermore, the up-regulation of the intrarenal RAS, hypothetically mediated by local ischemia, is associated with increased expression of Angiotensin Converting Enzyme (ACE) in proximal tubules and peri-tubular interstitium. And thus, ACE cleaves angiotensinogen and bradykinins creating an imbalance in favor of the former with the consequent stimulation of proliferation, fibrosis and salt retention (Carlos, 2003).

Hypertension is highly prevalent in CKD, being related with the level of renal function, the cause of the kidney disease and the age of the patient. Patients with vascular disease, diabetes and polycystic kidney disease (PKD) are more prone to be hypertensive (Ridao *et al.*, 2001). Guyton proposed that auto-regulation could be responsible for the secondary increase of the peripheral resistance in the presence of blood volume expansion, as it occurs in CKD (Guyton *et al.*, 1980). Again, the relevance of the RAS, in physiological terms, is based on its capacity to regulate arterial pressure and sodium balance. When the blood pressure or perfusion fall, or the sympathetic activity increases, the juxtaglomerular cells secrete renin, which cleaves angiotensinogen, leading to an increase in angiotensin II levels. This octapeptide is a powerful

vasoconstrictor and stimulates the production of aldosterone, which, in turn, increases renal sodium reabsorption, and closes the regulatory feedback loop. However, if the blood volume is normal, the increased activity of the RAS produces an abnormal rise in the blood pressure. Only a small proportion of CKD patients have a measurable increase of the RAS (Acosta, 1982). Furthermore, there is a greater production of local and intra-renal AII which then exacerbates systemic hypertension (Acosta, 1982). The rising incidence of CKD has been attributed to an aging population and increases in hypertension, diabetes, and obesity within the U.S population (Leticia and Charlotte, 2012). Hypertension is reported to occur in 85% to 95% of patients with CKD stages 3–5 (Rao *et al.*, 2008), irrespective of the origin. The relationship between hypertension and CKD is cyclical. Uncontrolled hypertension is associated with a more rapid progression of CKD and is the second leading cause of ESRD in the U.S (Botdorf *et al.*, 2011; Segura *et al.*, 2011). Progressive renal disease can however, exacerbate uncontrolled hypertension due to volume expansion and increased systemic vascular resistance.

Africa is not an exception to hypertension associated with CKD as studies have reported an increasing trend of chronic renal failure in both outpatients and inpatients in Africa with hypertension (Plange-Rhule *et al.*, 1999; Lengani *et al.*, 1994). In a 6-year study of patients with end-stage renal disease (ESRD), based on South African Dialysis and Transplant Registry statistics, hypertension caused ESRD in 4.3% of whites, 34.6% of blacks, 20.9% of mixed ethnic group, and 13.8% of Indians (Naicker, 2003), and a prevalence of proteinuria of 12.4% in a randomly selected population in the Democratic Republic of Congo (Sumaili *et al.*, 2009). In a “high-risk” population in the same country with diabetes, hypertension, HIV infection, or obesity, the prevalence of CKD was 36% (Sumaili *et al.*, 2009). In Nigeria, 10.4% of hypertension patients had an eGFR <60 mL/min (Afolabi *et al.*, 2009) while in Burkina Faso,

117 out of 317 (44%) patients with hypertension who were hospitalized had chronic renal failure (Lengani *et al.*, 1994). In Ghana, hypertension is a common cause of CKD and mortality. An autopsy study showed that hypertension was a major cause of end-stage renal failure in Ghana, accounting for 42% cases (Matekole *et al.*, 1993). A prevalence of CKD of 46.9% in outpatients with hypertension in Accra has been reported (Osafo *et al.*, 2011). Although multiple guidelines have been discussed on the importance of lowering blood pressure (BP) to slow the progression of renal disease and reduce cardiovascular morbidity and mortality (Chobanaian *et al.*, 2003; American Diabetes Association 2012), hypertension and CKD continue to increase worldwide that puts a serious burden on scarce economic resources and a threat to the survival of the population.

2.3. Fibroblast Growth Factor 23 (FGF23)

According to the ADHR consortium (2000) and Shimada *et al* (2001), FGF23 was originally discovered by positional cloning of the gene responsible for autosomal dominant hypophosphatemic rickets, a condition in which elevated serum levels of active FGF23 caused hypophosphatemia that resulted in rickets or osteomalacia. Fibroblast growth factor 23 is secreted into the bloodstream and thereafter, it acts as a hormone (Larsson *et al.*, 2003; Gattineni *et al.*, 2009). The physiological effects of FGF23 are mediated by FGF receptors (FGFRs), which are tyrosine kinases encoded, by four distinct genes (FGFR1–FGFR4) (Dailey *et al.*, 2005; Yu *et al.*, 2005). *In vitro* and *in vivo* studies have shown that FGF23 interacts with all four FGFRs (Gattineni *et al.*, 2009; Li *et al.*, 2011). Despite the presence of FGFRs everywhere, the target organs of FGF23 are limited to the kidney and parathyroid (Ben-Dove *et al.*, 2007) which expresses the FGF23 receptor, Klotho (Larsson *et al.*, 2004; Yu *et al.*, 2005). Klotho is also shed from cell surfaces by proteolytic cleavage and is released into circulation and serves as a

hormone with phosphaturic effects that are independent of FGF23 (Hu *et al.*, 2010). The primary target of FGF23 is the FGF receptor-Klotho complex in the kidney, thus FGF23 induces urinary phosphate excretion by decreasing expressions of the type IIa and IIc sodium-dependent phosphate co-transporters (NPT2a and 2c) in the renal proximal tubule (Yan *et al.*, 2005).

Furthermore, FGF23 decreases dietary absorption of phosphate through suppression of circulating concentrations of 1, 25(OH) 2D by inhibiting renal expression of the 1, 25-dihydroxyvitamin D-synthesizing CYP27B1 (1- α -hydroxylase) and stimulating expression of catabolic 24-hydroxylase (Shimada *et al.*, 2004). This mechanism is supported by results of studies that demonstrated that treatment with FGF23-neutralizing antibody prevents a decrease in serum 1, 25 (OH) 2D in rats with progressive CKD (Hasegawa *et al.*, 2010). Fibroblast Growth Factor-23 is present in human circulation in several major forms; the hormonally active intact FGF23 (iFGF23), inactive C-terminal (cFGF23) and an N-terminal fragment (nFGF23). It is observed that iFGF23 is the biologically active form that mediates the direct effects of phosphate and vitamin D metabolism; when in excess, cFGF23 has been demonstrated to inhibit the action of iFGF23 (Goetz *et al.*, 2007).

2.3.1 Regulation of FGF23

There are differences in how FGF23 is regulated in normal subjects and subjects with metabolic bone diseases as against those with renal insufficiency and renal failure. What regulates the very high blood levels seen in renal disease is not clear. While levels of FGF23 in late renal disease correlate with serum phosphate, FGF23 levels begin to rise before there is an increase in serum phosphate (and/or PTH), in early stages of renal failure, suggesting that initially there is a primary phosphate- and PTH-independent regulation of FGF23 in mild renal insufficiency

(Isakova *et al.*, 2011). Some studies have postulated that the hormone could be regulated by vitamin D and parathyroid hormone (PTH). In one such study, it was reported that vitamin D controls FGF23 production (Liu *et al.*, 2006). In another study, specific disruption of vitamin D receptors in bone cells decreased FGF23 production (Masuyama *et al.*, 2006), suggesting that the vitamin D receptor element in the FGF23 promoter plays a physiologic role. In line with these findings, vitamin D receptor null mice showed undetectable FGF23 levels (Shimada *et al.*, 2005). In a clinical study, intravenous active vitamin D injection significantly increased serum FGF23 levels in dialysis patients with secondary hyperparathyroidism (Nishi *et al.*, 2005). These findings explain the regulatory feedback loop that is formed between FGF23 and vitamin D. Again, Iron plays a potential role in the regulation of FGF23. Durham *et al* (2007) detected an elevated C-terminal FGF23 level in subjects with low ferritin .They made the observation that in subjects who were most likely iron deficient, there were elevated levels of cFGF23, but normal levels of iFGF23, thus offering the first suggestion of a role for iron in FGF23 metabolism and hinting that FGF23 processing may be involved in regulation of iFGF23 levels. Subsequent work showed that iron infusions leads to FGF23-mediated hypophosphatemia (Schouten *et al.*, 2009, Shimizu *et al.*, 2009).

2.3.2 FGF23 and Hypertension

The causes and origin of hypertension have been reported by several researchers. The latest addition to the causes of hypertension and hence heart diseases and kidney damage is the hormone FGF23. According to Olena *et al* (2013), FGF23 controls renal sodium excretion and therefore blood pressure. The researchers reported that FGF23 has a sodium retention effect and thus controls the reabsorption of filtered sodium in the kidneys. This was demonstrated in mice

in which mice lacking FGF23 excreted higher amounts of sodium in their urine and had a low blood pressure while those with high FGF23 level showed high levels of sodium in their blood, low urinary sodium and a high blood pressure. The researchers hypothesized that elevated level of FGF23 could put a strenuous strain or pressure on the heart and increases the chances of cardiovascular diseases. In a community based cross-sectional study, Gutierrez *et al* (2011) reported that higher plasma level of FGF23 was independently associated with history of hypertension and that history of hypertension was an independent predictor of higher plasma concentrations of FGF23.

2.3.3 FGF23 and Chronic Kidney Disease

The association and level of FGF23 in CKD have been reported in several studies. Researchers have identified FGF23 as a risk factor of CKD progression. In patients with non-diabetic CKD, higher levels of cFGF23 and iFGF23 were independently associated with incident ESRD (Danilo *et al.*, 2007). Fibroblast growth factor 23 is strongly associated with mortality in patients with ESRD (Isakova *et al.*, 2011; Gutierrez *et al.*, 2011), and in patients with CKD where it is also linked to progression toward renal failure (Tonelli *et al.*, 2005). It has also been linked to the development of left ventricular hypertrophy, vascular dysfunction, and vascular calcification (Gutierrez *et al.*, 2009). Disordered phosphate balance with elevated circulating levels of fibroblast growth factor 23 (FGF23) is an early and pervasive complication of CKD. According to Wolf (2012), chronic kidney disease is likely the most common cause of chronically elevated FGF23 levels, and the clinical condition in which levels are most markedly elevated.

In a mild to moderate chronic kidney disease study (Danilo *et al.*, 2007) in patients with non-diabetic CKD, elevated FGF23 was reported in magnitude as CKD progresses from stage 2 to stage 5. In that study, in the presence of progressive CKD, serum FGF23 levels increase in parallel with the deterioration of renal function and the increase of serum phosphate and PTH concentrations. In similar studies, Gutierrez *et al* (2005) found an increase in FGF23 serum levels in early stages of CKD although the exact CKD stage when FGF23 levels first became significantly elevated differed across studies. This pathophysiological sequence is supported by an analysis of mineral metabolites in participants with CKD stages 2–4 in the prospective Chronic Renal Insufficiency Cohort (CRIC) study in which C-terminal, FGF23 levels were elevated in stage 2 and most stage 3–4 patients, and an elevated FGF23 level was more prevalent than elevated PTH or serum phosphate at all levels of eGFR (Isakova *et al.*, 2011). These findings may explain the basis for using FGF23 as a biomarker of CKD.

2.3.4 Assay methods of FGF23

There are currently two commercially available enzyme-linked immunosorbent assays (ELISA) for measurement of FGF23 concentration, namely the Kainos “Intact” FGF23 ELISA (Kainos Laboratories, Inc., Tokyo, Japan) and the Immotopics “C-terminal” FGF23 ELISA (Immutopics, Inc., CA, USA). The current assays both measure the full-length molecule, and the “C-terminal” assay from Immotopics, which detects both the intact molecule and C-terminal fragments as well. Though there is initial concern as to whether values obtained with the Immotopics C-terminal assay tends to overestimate the true amount of biologically active FGF23 in circulation. Available data suggests that almost all detectable FGF23 in advanced CKD circulates in the full-length, biologically active form of the molecule and thus measurements obtained with the Kainos

“intact” assay and the Immutopics “C-terminal” assay reflect the same circulating moiety (Shimada *et al.*, 2010). There are differences among these FGF23 assays. The measurements obtained with the 3 assays differ, not only in their absolute numerical readouts but also in the unit of measure reported (picograms per milliliter vs relative units per milliliter), and not much information is available on the relationship between the results obtained with the 2 Immutopics assays and those obtained with the Kainos assay. Studies have yet to determine the possibility of converting results obtained by one assay into results obtained by another; compare the accuracy and reproducibility of the three assays, and the reference interval for each assay. Thus, standardization of all 3 assays is necessary before they can be considered ready for routine clinical use and incorporation into clinical practice guidelines. The Intact ELISA uses two antibodies that recognize the N-terminal and C-terminal regions and therefore only recognizes the full, intact FGF23 hormone prior to proteolytic cleavage. However, the two antibodies used in the C-terminal ELISA detect epitopes within the C-terminal region and therefore recognizes both the intact hormone and the C-terminal fragment.

2.4 Biochemical Markers of Renal Disease

Biochemical markers play a fundamental role in accurate diagnosis and also for assessing risk and adopting therapy that improves clinical outcomes. Over the years, research and utilization of biomarkers has evolved substantially. In 2001, the National Institute of Health (NIH) defined a biomarker as a characteristic that is objectively measured and evaluated as an indicator of normal biological, pathologic processes, or pharmacologic responses to a therapeutic intervention (Ramachandan, 2006). As markers of renal function creatinine, urea, uric acid and electrolytes are for routine analysis whereas several studies have confirmed and consolidated the usefulness

of markers such as cystatin C, β -Trace Protein. Plasma concentrations of the waste substances of creatinine and urea as well as electrolytes are used to determine renal function. These measures are adequate to determine whether a patient is suffering from kidney disease. Though blood urea nitrogen (BUN) will not be raised above the normal range until 60% of total kidney function is lost, the only abnormality in the plasma early in the process may be an increase in the plasma levels of urea and creatinine, which do not occur until 50-70% of the nephrons are destroyed (Burtis *et al.*, 2006). As the disease progresses, reflected by a further fall in the GFR, other analytes such as potassium, sodium, hydrogen ions, phosphate, and urate appear in the blood in increasing amounts (Koay *et al.*, 1999).

2.4.1. Microalbuminuria

Microalbuminuria is defined as urine albumin-creatinine ratio (UACR) on spot urine sample of 2.5-25mg/mmol in males and 3.5-35mg/mmol in females (Chadban *et al.*, 2003). Presence of detectable amount of albumin in urine is an early indication of deteriorating kidneys. Creatinine and albumin in urine are measured for calculating the UACR from the relation: albumin (mg/dl)/creatinine (mmol/dl). The albumin-to-creatinine measurement is used to detect kidney disease in people at high risk, especially those with diabetes or hypertension (Koay *et al.*, 1999). Similarly, microalbuminuria (urinary albumin excretion of 30-300 mg/24 hours), according to the Canadian Diabetes Association (2003), is one of the earliest markers of nephropathy with the presence of minimal amount of albumin in the urine. Microalbuminuria may progress over a number of years to overt nephropathy characterized by the presence of larger amounts of albumin in urine. This is called macroalbuminuria (urinary albumin > 300 mg/24 hours). The presence of macroalbuminuria indicates more serious kidney disease.

Studies have shown that presence of microalbuminuria is reversible with interventions to tightly control blood sugar and blood pressure and that microalbuminuria may sometimes resolve on its own (Yuyan *et al.*, 2004). Specific medications including angiotensin converting enzyme inhibitors and angiotensin receptor blockers have been shown to halt and reverse the presence of microalbuminuria and delay the progression to ESRD and the need for dialysis. In some cases a combination of both agents is indicated to protect the kidneys (Ephraim *et al.*, 2015)

Microalbuminuria is measured on spot early morning urine collections, timed urine collections or as a ratio of albumin to creatinine in urine (ACR). The ACR is the preferred method as it does not require early morning or timed collections, it correlates with the 24-hour urine values over a large range of proteinuria, it is cheap to perform, and repeat values can be easily obtained to be certain ascertain that microalbuminuria, if present, is persistent (Yuyan *et al.*, 2004). A patient is considered to have nephropathy if ACR is elevated above 2.0 mg/mmol in men or 2.8 mg/mmol in women. Microalbuminuria is associated with increase risk of kidney disease and cardiovascular disease in patients with diabetes and hypertension. Microalbuminuria reflects vascular damage and appears to be a marker of early arterial disease and endothelial dysfunction (Yuyan *et al.*, 2004).

2.4.2 Creatinine

Serum creatinine concentration is maintained by the balance between its generation and excretion by the kidneys. Levels are affected by factors that influence the generation, glomerular filtration, and tubular secretion of serum creatinine. There is considerable variation in the excretion of creatinine based on individual patient factors, time, and method of testing. Since creatinine is generated in a steady manner and can be measured very simply from blood samples,

it has become a useful test to estimate glomerular filtration rate (GFR), a measurement of kidney function. Creatinine is a breakdown product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body depending on muscle mass (Yuegang *et al.*, 2008). Creatinine is commonly used to assess kidney function. The normal creatinine clearance test value is 110-150ml/min in male and in female it is 100-130ml/min (Corbett, 2008). The National Kidney Disease Education Program recommends calculating glomerular filtration rate from serum creatinine concentration (Miller *et al.*, 2005). The creatinine clearance test is often used to monitor the progression of renal disease and a diagnosis of renal failure is usually suspected when serum creatinine is greater than the upper limit of the “normal” interval. In chronic renal failure and uremia, an eventual reduction occurs in the excretion of creatinine by both the glomeruli and the tubules (Edmund and David, 2006). Creatinine values may vary as its generation may not be simply a product of muscle mass but influenced by muscle function, muscle composition, activity, diet and health status (Banfi and Del, 2006). The elevated values are also seen in muscular dystrophy paralysis, anemia, leukemia and hyperthyroidism. The decreased values are noticed with glomerulonephritis, congestive heart failure, acute tubular necrosis, shock, polycystic kidney disease, and dehydration (Edmund and David, 2006).

2.4.3 Urea

Urea is major nitrogenous end product of protein and amino acid breakdown, produced by liver and distributed throughout intracellular and extracellular fluid. In kidneys, urea is filtered out of blood by the glomerular and is partially being reabsorbed with water (Corbett, 2008). The most commonly determined clinical indices for estimating renal function is the concentration of urea in the serum. It is useful in differential diagnosis of acute renal failure and pre renal condition

where blood urea nitrogen–creatinine ratio is elevated above the normal (Mitchell and Kline, 2006). Urea clearance is a poor biomarker of glomerular filtration rate as its overproduction rate depends on several non renal factors, including diet and urea cycle enzymes. Increased blood urea nitrogen (BUN) is associated with kidney disease or failure, blockage of the urinary tract by a kidney stone, congestive heart failure, dehydration, fever, shock and bleeding in the digestive tract (Corbett, 2008). High BUN levels can sometimes occur during late pregnancy or result from eating large amounts of protein-rich foods. A BUN level higher than 100 mg/dL indicates severe kidney damage whereas decreased BUN is observed in fluid excess. Low levels are also seen in trauma, surgery, opioids, malnutrition, and anabolic steroid use (Pagana and Kathleen, 2002).

2.4.4 Cystatin C

Cystatin C is a non-glycosylated low molecular weight protein. Cystatin C is produced by all nucleated cells at a constant rate and is freely filtrated by the glomeruli and completely catabolized in the proximal tubules. The concentration of serum Cystatin C is mainly determined by glomerular filtration, which makes Cystatin C an endogenous marker of glomerular filtration rate (Randers and Erlandsen, 1999). In one study (Dharnidharka *et al.*, 2002), the researchers found Cystatin C to be superior to serum creatinine as a marker of glomerular filtration rate. Other studies have affirmed this with similar results when compared with other markers such as a 1-microglobulin and β 2-microglobulin (Guido *et al.*, 2002). Cystatin C has been found more useful for detecting early renal impairment in both type 1 and type 2 diabetic patients (Laura *et al.*, 2007). Similarly, Cystatin C was also found to be associated with mild kidney dysfunction with increased risk for cardiovascular events, peripheral arterial disease and heart failure (Shlipak *et al.*, 2005).

2.4.5 Proteinuria

Clinically the appearance of significant amount of protein in urine is one of the earliest sign of almost all renal diseases. Estimation of proteinuria helps in differentiating between tubulointerstitial and glomerular diseases and also to follow the progress of renal disease and to assess the response to therapy. Normally excretion in most healthy adults is between 20-150 mg of protein in urine over 24 hrs. Proteinuria more than 3.5 gm/day is taken to be diagnostic of nephrotic syndrome. Panels of protein measurement including albumin, α 2-macroglobulin, immunoglobulin G and α 2- microglobulin have been employed in differential diagnosis of prerenal and post renal disease. It has been recommended the use of the protein/creatinine ratio as an Index of Quantitative Proteinuria in 24 hour urine collection (Sandeep *et al.*, 2004). The use of the clearance of haptoglobin, in particular provided valuable diagnostic information in cases in which the routine methods gave borderline values for the index of proteinuria (Tishkov *et al.*, 1978). During pregnancy proteinuria assay in 24 hour urine sample is performed. One of the investigations for proteinuria is semi-quantitative dipstick urinalysis as this method is relatively low cost and easily performed (Helena and Jason, 2004). In pregnancy automated dipstick urinalysis is a more accurate screening test for the detection of proteinuria than visual testing. The finding of dipstick proteinuria should be confirmed by either a 24 hour urine collection or a protein-creatinine ratio (Jason *et al.*, 2005).

2.4.6 Estimated Glomerular Filtration Rate (GFR).

According to the National Kidney Foundation (2012), estimated GFR (eGFR) gives indication of functioning kidney mass. Use of estimated GFR derived from creatinine estimations is more useful especially because of the problems with measuring creatinine clearance. GFR is estimated

by measuring the clearance of exogenous filtration markers such as iothalamate, iohexol, and inulin. However, these methods are expensive and require exposure to radiation and compliance with strict regulatory guidelines, and thus have limited use in the routine laboratory settings. On the other hand, creatinine is freely filtered, has minimal tubular secretion and absorption, is simple and inexpensive to measure from random blood samples, and has relatively good accuracy. It has therefore become a valuable clinical tool for estimating GFR. A rise in serum creatinine is used in clinical practice as a marker of reduced GFR. It varies inversely with GFR, but the relationship is not linear. GFR can be estimated by measuring creatinine clearance using serum creatinine levels and a timed urine specimen. The use of serum creatinine as an indirect filtration marker is limited by its biological variability, bias and non-specificity affecting creatinine measurement, medication effects, nutrition, and by the alterations in circulating serum creatinine produced by non-renal disease states.

2.4.6.1 Estimation or measurement of GFR

There are a number of equations that are used to estimate GFR. Three common equations that are used are the CKD-EPI creatinine equation, the Modification of Diet in Renal Disease (MDRD) Study equation, and Cockcroft and Gault equation. All three of these equations use serum creatinine in combination with age, sex, weight or race to estimate GFR and, therefore, improve upon several of the limitations with the use of serum creatinine alone. Estimated GFR (eGFR) equations, based on serum creatinine, are generally utilized for the systematic staging of chronic kidney disease (CKD). However, the utility of the estimation formulas of GFR in the acute clinical setting has not yet been validated in Ghana (Osafo *et al.*, 2011). The estimation of GFR by serum creatinine differs between healthy people and patients with CKD because of

differences in GFR range and creatinine production between these two populations. As a result of these confounding factors, there is a risk of overestimating the GFR, and the magnitude of the overestimation is not predictable (Levey *et al.*, 2007). The equations used for estimating GFR are mainly for staging of CKD. Comparative study of estimated creatinine clearance in critically-ill patients by Cockcroft-Gault, Modification of Diet in Renal Disease (MDRD), and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations showed a high degree of variability.

2.4.6.1.1 The MDRD equation

Glomerular Filtration Rate is estimated mostly from the Modification of Diet in Renal Disease (MDRD) GFR equation (Levey *et al.*, 2009). It performs well in populations with a low range of GFR and often out-performs the Cockcroft-Gault equation. This equation has several limitations, including age, disease state, and considerable variations in the standardization of the serum creatinine assays. The MDRD GFR formula generally offers reliable data for the calculation of drug doses. In peritoneal dialysis patients, creatinine clearance estimated from the MDRD equations may accurately approximate measured 24-hour urine and dialysate creatinine clearance; it should not be used to assess small-solute removal or adequacy in individual patients or to predict outcome in any cohort of patients over narrow ranges of limited clearance (Levey *et al.*, 2009).

MDRD: $GFR (ml/min/1.73m^2) = 30849 \times \text{serum creatinine } (\mu\text{mol/L})^{-1.154} \times \text{Age (years)}^{-0.203} \times 1.212 \text{ (if black)} \times 0.742 \text{ (if female)}$

The original MDRD equation was developed using the alkaline picrate method and is subject to error if serum creatinine measurements obtained by other methods are used. For this reason, the abbreviated MDRD equation has been revised to produce the IDMS-traceable abbreviated MDRD equation. This equation can be used with different creatinine measurement methods once the measurements have been recalibrated and standardized to IDMS.

2.4.6.1.2 Cockcroft- Gault Equation

The Cockcroft-Gault equation is an alternative equation that estimates creatinine clearance, but has the limitation that the serum measurements on which it was based were not standardized using IDMS. The Cockcroft-Gault formula was developed in 1976 with a wide range of renal function (Levey *et al.*, 2009). It uses age, the inverse of serum creatinine, and lean body weight to estimate creatinine clearance in milliliters per minute. The inclusion of the weight factor is intended to adjust for muscle mass, a determinant of serum creatinine concentration. This implies that in clinical situations in which a change in weight is not the result of a similar change in muscle mass (e.g., edematous states, pregnancy, third spacing, overweight, obesity), the weight factor will adversely affect the performance of this formula. Because the original mathematical model was derived from data obtained predominantly in a male population, an arbitrary adjustment for female sex by a factor of 0.85 was incorporated. This equation has become popular because of its simple mathematical formulation and bedside applicability. It is important to note that this formula estimates creatinine clearance; this is known to overestimate GFR because of tubular secretion of creatinine, which is not adjustable.

CrCl (ml/s) = { (140-age) × weight(kg) × 0.85 (if females)} ÷ {(48816 × SCr (μmol/L))}, Age in years, where CrCl =creatinine clearance, SCr=serum creatinine.

2.4.6.1.3 Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI)

The CKD-EPI equation was developed in 2009 using a diverse population estimate GFR from serum creatinine, age, sex and race. The CKD-EPI equation is as accurate as the MDRD Study equation in the subgroup with estimated GFR less than 60 mL/min/1.73 m² and substantially more accurate in the subgroup with estimated GFR greater than 60 ml/min/1.73 m². It is the recommended equation in the Kidney Disease: Improving Global Outcomes (KDIGO), Chronic Kidney Disease Guidelines. The CKD-EPI equation is expressed as a single equation as indicated by the Ann Intern Med, 2009. One study has shown that the CKD-EPI equation is more accurate than the MDRD equation for estimating GFR, especially for values >60 mL/min/1.73m². However, elderly people and black people with higher levels of GFR and ethnic minorities other than black people were not well represented in the study. In one meta-analysis of data from 1.1 million adults comparing the CKD-EPI with the MDRD equation, 24.4% and 0.6% of participants from general population cohorts were reclassified to a higher and lower estimated GFR category, respectively, by the CKD-EPI equation (Levey *et al.*, 2009). The prevalence of CKD stages 3 to 5 (estimated GFR <60 mL/min/1.73 m²) was reduced from 8.7% to 6.3% (Levey *et al.*, 2009). **$GFR=141 \times \min(Scr/k, 1)^\alpha \times \max(Scr/k, 1)^{-1.209} \times 0.993^{Age} \times 1.159 \times 1.018(\text{if female})$** where; Scr=standardized serum creatinine in mg/dL, k=0.9 and 0.7 for males and females respectively $\alpha=-0.329$ and -0.411 for females and males respectively, min= minimum of Scr/k or 1, max= maximum of Scr/k or 1.

2.5 Staging of Chronic Kidney Disease

2.5.1. The K/DOQI method of classifying/ staging CKD

The Kidney Disease Outcomes Quality Initiative (K/DOQI) of the National Kidney Foundation (NKF) defines chronic kidney disease as either kidney damage or a decreased glomerular filtration rate (GFR) of less than 60 mL/min/1.73 m² for 3 or more months. Whatever the underlying etiology, the destruction of renal mass with irreversible sclerosis and loss of nephrons leads to a progressive decline in GFR. The different stages of chronic kidney disease form a continuum in time. In 2002, K/DOQI published its classification of the stages of chronic kidney disease based on estimated GFR as follows (K/DOQI, 2002):

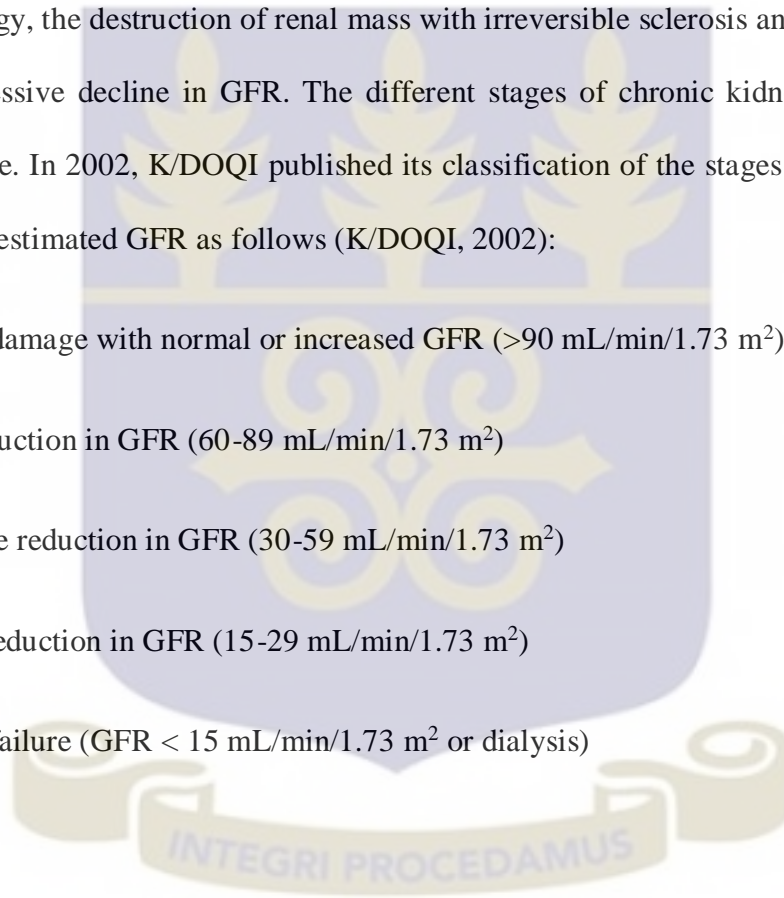
Stage 1: Kidney damage with normal or increased GFR (>90 mL/min/1.73 m²)

Stage 2: Mild reduction in GFR (60-89 mL/min/1.73 m²)

Stage 3: Moderate reduction in GFR (30-59 mL/min/1.73 m²)

Stage 4: Severe reduction in GFR (15-29 mL/min/1.73 m²)

Stage 5: Kidney failure (GFR < 15 mL/min/1.73 m² or dialysis)



CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1. Study Site:

The study was conducted at the Holy Family Hospital in the Brong-Ahafo Region of Ghana. The Hospital is located in a heterogeneous populated area, Techiman municipality. The hospital has a bed capacity of 167 beds and is owned by the Catholic Diocese of Techiman and a member of the National Catholic Health Services (Sammy *et al.*, 2015). It serves as a referral facility that provides primary, secondary, and tertiary healthcare needs for its surrounding districts and regions such as the Upper West and East Regions. Situated between North and West of Ghana, it serves at least 15,000 patients a year. The hospital has a well coordinated outpatient hypertension unit for managing hypertension cases and serves as a referral centre for surrounding districts to the Northern regions of Ghana. Consequently the hospital receives a lot of hypertension cases of at least 355 yearly (Sammy *et al.*, 2015).

3.2. Study Design and Selection of subjects

The study was a hospital based case control study in which subjects were selected using purposive sampling to differentiate subjects who were consecutive hypertension and normotension patients attending the hypertensive clinic. Hypertension was defined as a diagnosis of blood pressure greater than 140/90mmHg, and on anti-hypertensive drug(s). The normotensives were qualified blood donors who had come to the blood bank of the hospital to donate blood.

3.3 Sample Size Determination

The sample size (n) was determined using the Kish-Leslie formula; $n = \frac{t^2 \times p(1-p)}{m^2}$; where t is the confidence level (CI) at 95%, p is the probability of the general population with hypertension in Ghana, m is the margin of error. Using 28% proportion of hypertension in the adult population in Ghana, then p was taken to be 28% (0.28); t = 1.96 at 95% CI, m = 0.05(5%).

Thus, $n = [1.96^2 \times 0.28 (1-0.28)] \div 0.05^2$

$$(3.8416 \times 0.28) \div 0.0025$$

N = 309

Hence, a sample size of 309 was obtained for the purpose of this study. However, 146 consented subjects finally took part in the study.

3.4 Inclusion Criteria

The researcher recruited eligible adult (> 18years) outpatients receiving medical treatment at the hospital during the study period. Patients diagnosed with high blood pressure or on anti-hypertensive drugs for at least a year were included as well as blood donors without high blood pressure or on anti-hypertensive drugs.

3.5 Exclusion Criteria

The study excluded respondents who were on cheese products and cola nuts that can increase FGF23 plasma levels. Female respondents on oral contraceptive pills, obese patients, diabetics as well as pregnant women were also excluded from the study. Individuals that did not consent to the study were also excluded.

3.6 Ethical Clearance

The study was approved by the Ethical and Protocol Review Committee of the University of Ghana, College of Health Sciences (**REF: MS-Et/M.3-P3.2/2015-2016**). Written informed consent (Appendix 1) was obtained from all respondents after taking them through the essence of the study. Approval was again obtained from the Holy Family Hospital's Ethics and Research Committee prior to the study. All information about the respondents was treated as confidential, with files containing the data made accessible only to the researcher. Data files were coded in a personal computer locked with a password.

3.7 Data Collection

The data for this study was demographic, anthropometric data (**Appendix II**), blood and urine samples.

3.7.1 Demographic and Anthropometric data

Information on demographic data (duration of condition, drug of choice, age and sex) were obtained using a structured pre-tested questionnaire (**Appendix II**) administered to the consented subjects while the anthropometric data (weight and blood pressure) were obtained from the patients' folders or medical records as recorded in the morning by the attending staff nurse as blood and urine samples of the subjects were taken. The type of drug or medication used to manage hypertension and CKD subjects was also obtained from the medical record folder. A certified trained nurse used a mercury sphygmomanometer (Accoson, England) with a standard or a large cuff, appropriate to the patient's size, to measure blood pressure after patients had rested for 5 minutes, in accordance with recommendations of the American Heart Association Council on High Blood Pressure Research.

3.7.2. Blood sample Collection and Processing

Five (5) mL of venous blood sample was taken from each respondent who had fasted for eight (8) to twelve (12) hours. Two (2) and three (3) ml of the blood was dispensed into serum separator and EDTA-K3 tubes respectively. The serum separator samples were centrifuged at 3000r/min for 5 minutes (Cheesbrough, 2000) and the serum was separated into Eppendorf tubes and stored at 2-8°C while plasma obtained from the EDTA-K3 anticoagulated blood was stored at -20°C prior to analysis.

3.7.3 Biochemical Analysis of Blood Sample

The serum was thawed and analyzed for creatinine, urea, calcium and phosphate. Serum creatinine was determined by the Jaffe reaction method (Bartels *et al.*, 1971), urea by the Oxime method (Kanai, 2007) and calcium determination by o-cresolphthalein-complexone (CPC) method (Barnett *et al.*, 1973) using Mindray BS-130 automated analyzer. By using the photometric UV test method (Gamst *et al.*, 1980), phosphate was measured in with URIT-810 automated analyzer. Sodium was estimated using Caretium Ion Selective Electrolyte Analyzer. Plasma concentration of FGF-23 was measured using the ELISA method (Kono Biotech Co., Ltd), a double-antibody sandwich enzyme-linked immunosorbent one-step assay process in line with the manufacturer's guide and procedures. According to the manufacturer's instruction, FGF-23 concentration of 31.2 – 2000 pg/ml is regarded as normal range for healthy adults.

3.7.4 Urine sample collection and processing

Each eligible respondent was given a sterile urine container and educated on collecting midstream urine by clean-catch technique. Samples collected were appropriately labeled and stored at 2-8°C prior to analysis.

3.7.5 Biochemical Analysis of Urine

Urine albumin and creatinine were measured using the bromocresol green (Dumas *et al.*, 1971) and Jaffe reaction (Bartels, 1971) methods respectively using Mindray BS-130 automated analyzer. For urine creatinine estimation, the researcher diluted the urine by 1 in 49ul according to the manufacturer (Fortress Diagnostics, England).

3.8. Estimation of GFR and urine Albumin –Creatinine Ratio

Urine albumin-creatinine ratio (UACR, mg/mmol) was derived from the relation: $UACR \text{ (mg/mmol)} = \text{urine albumin (mg/dl)} / \text{urine creatinine (mmol/dL)}$. Microalbuminuria was defined as UACR on spot urine sample of 2.5-25mg/mmol in males and 3.5-35mg/mmol in females (Chadban *et al.*, 2003). The estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI equation. The K/DOQI staging criteria was applied to identify the various stages of CKD in patients with defined CKD.

3.9. Statistical Analysis

Analyses and statistical procedures were carried out using the Statistical Package for Social Sciences program (SPSS, version 21.0 for Windows) and GraphPad prism 5 for windows. Results are expressed as means \pm SD. Unpaired t-test was used to compare mean values of continuous variables i.e FGF23, calcium, sodium, phosphate, urea and creatinine whiles Chi-

Square (χ^2) test statistic was used for all categorical variables (GFR, CKD and hypertension grades). ANOVA was used to compare the difference in the mean values of more than two groups. Person's correlation analysis was used to determine the relationship between FGF23, anthropometric and demographic characteristics among Hypertensives and Hypertensives with CKD. Logistic regression was used to determine odds for increase in FGF23 hormone in hypertensives. For all statistical comparisons, a $P \leq 0.05$ was considered as statistically significant.



CHAPTER FOUR

4.0 RESULTS

4.1: Demographic and clinical characteristics of Hypertensives (HTP) and Normotensives (NTP)

The proposed 309 expected to be used for this study was reduced to 146 due to the high cost of FGF23 ELISA KIT and approved consent. Those with hypertension were those with sustained and established high blood pressure who were undergoing periodic management, treatment and monitoring at the Holy Family Hospital, Techiman. Out of the 146 respondents, 25 of them were excluded because of incomplete data provided including urine samples. As shown in Table 4.1, 86 respondents had hypertension that sought periodic management and monitoring at the hospital while 60 of the respondents were healthy blood donors (normotensives) with no history of hypertension. The mean age of subjects with hypertension was 62.05 ± 12.06 and without hypertension were 29.12 ± 6.57 years. In all, 34 males and 71 females consented and provided urine and blood samples. There was significant difference in systolic ($P < 0.0001$) and diastolic ($P = 0.01$) blood pressure between the subjects with hypertension and those with no hypertension. All biochemical analytes (urea, calcium, sodium, phosphate, urine albumin creatinine ratio, estimated GFR, and FGF23) showed significant difference ($P < 0.05$). However, there was no significant difference in the creatinine concentration ($\mu\text{mol/L}$) in both study populations with mean concentrations of 100.96 ± 34.45 and 101.57 ± 17.85 ($P > 0.05$) in both respondents with and without hypertension respectively.

4.2: Biochemical characteristics, estimated glomerular filtration rate (eGFR) and stages of Chronic Kidney Disease among Hypertensives stratified by gender

Table 4.2 shows the biochemical characteristics of subjects with both hypertension and chronic kidney disease. The demographic (age, sex), anthropometric (systolic blood pressure, diastolic blood pressure, and weight), and biochemical (urea, creatinine, sodium, phosphate, calcium, FGF23, UACR) did not show significant differences between males and females. The stages of CKD in both males and females did not also show any statistical difference ($P>0.05$). While respondents had a mean UACR of the respondents was 4.83 ± 0.78 , the mean concentration of FGF23 (pg/ml) was 2193.35 ± 1683.57 . Defined by high UACR, urea, creatinine and estimated GFR, 27.9% of all respondents with hypertension had chronic kidney disease (CKD). Among those with CKD, 9 (10.5%) had stage 2 (eGFR=60-89 while 13(15.1%) had stage 3 (e GFR= 30-59) of CKD. Again, 2(2.3%) had stage 4 (eGFR=15-29) of the disease. No stage 1 (eGFR \geq 90) of CKD was reported. Also, 46.5% of the respondents with hypertension had estimated GFR \geq 90 while 1.2% had an estimated GFR of 15-29 (Table 4.2).

4.3: Demographic and biochemical characteristics among Hypertensives and Hypertensives with CKD

Table 4.3 depicts a comparison of the biochemical characteristics of the study participants with hypertension and CKD. The least duration of hypertension was 1-5 years while the longer duration of hypertension was 16-20 years. In all, 41.9% of the respondents had hypertension for 1-5 years. Also, 41.7% had CKD for 1-5 and 41.7% had hypertension with CKD for 6-10 years. There was no significant difference in the duration of hypertension with and without CKD

($P=0.5$). Again, there was no significant difference in any of the drugs used between respondents with hypertension and CKD ($P=0.78$).

Table 4.1: Demographic and clinical characteristics of Hypertensives (HTP) and Normotensives (NTP)

Variable	HTP (n = 86)	NTP (n = 60)	P-value
Gender			<0.0001
Male	18 (20.9)	53 (88.3)	
Female	68 (79.1)	7 (11.7)	
Age (years)	62.05 ± 12.60	29.12 ± 6.57	<0.0001
Age group n (%)			<0.0001
<40	3 (3.5)	58 (96.7)	
40-49	9 (10.5)	1 (1.7)	
50-59	27 (31.4)	1 (1.7)	
60-69	20 (23.3)	0 (0.0)	
70-79	19 (22.1)	0 (0.0)	
≥ 80	8 (9.3)	0 (0.0)	
Weight (Kg)	63.73 ± 14.08	66.11 ± 9.69	0.259
Blood pressure (mmHg)			
SBP	144.71 ± 21.86	122.25 ± 9.33	<0.0001
DBP	82.95 ± 10.24	77.08 ± 5.31	<0.000
Phosphate (mmol/L)	3.21 ± 2.89	1.53 ± 0.31	<0.000
Sodium (mmo/L)	149.37 ± 13.00	139.50 ± 6.31	<0.000
Calcium (mmol/L)	6.61 ± 2.52	9.85 ± 1.66	<0.0001
FGF23 (pg/mL)	2193.35 ± 1683.57	657.67 ± 221.83	<0.0001
Urea (mmol/L)	8.45 ± 7.37	5.06 ± 0.75	0.001
Creatinine (μmol/L)	100.96 ± 34.45	101.57 ± 17.85	0.740
UACR	4.83 ± 0.78	0.34 ± 0.04	0.001
eGFR, mL/min/1.73 m²	69.91 ± 22.35	98.07 ± 19.66	<0.0001

Table 4.1 shows demographic and clinical characteristics of Hypertensives (HTP) and Normotensives (NTP); SBP: Systolic blood pressure; DBP: Diastolic blood pressure; FGF23: Fibroblast growth factor-23; UACR: Urine Albumin creatinine ratio; e GFR: Estimated glomerular filtration rate, n: number of respondents, p-value <0.05: for statistical significance

Table 4.2: Biochemical characteristics, estimated glomerular filtration rate (eGFR) and stages of Chronic Kidney Disease among Hypertensives stratified by gender

Variable	Male (n = 18)	Female (n= 68)	Total (n = 86)	P-value
Weight (Kg)	39.40 ± 9.66	63.67 ± 15.09	63.73 ± 14.08	0.942
Blood pressure (mmHg)				
SBP	144.33 ± 19.47	144.81 ± 22.58	144.71 ± 21.86	0.935
DBP	81.89 ± 8.95	83.23 ± 10.59	82.95 ± 10.24	0.623
Phosphate (mmol/L)	3.70 ± 0.89	3.08 ± 0.32	3.21 ± 2.89	0.420
Sodium (mmo/L)	152.63 ± 10.87	148.51 ± 13.44	149.37 ± 13.00	0.233
Calcium (mmol/L)	6.40 ± 2.60	6.66 ± 2.52	6.61 ± 2.52	0.695
FGF23 (pg/mL)	2421.39 ± 2056.43	2132.99 ± 1582.78	2193.35 ± 1683.57	0.521
Urea (mmol/L)	9.14 ± 7.86	8.27 ± 7.29	8.45 ± 7.37	0.659
Creatinine (µmol/L)	109.01 ± 31.67	98.83 ± 35.10	100.96 ± 34.45	0.268
ACR	5.73 ± 2.22	4.59 ± 6.59	4.83 ± 0.78	0.552
ACR				
High	7 (29.2)	17 (70.8)	24 (27.9)	0.243
Low	11 (17.7)	51 (82.3)	62 (72.1)	0.280
eGFR, mL/min/1.73 m²	73.22 ± 25.69	69.04 ± 21.50	69.91 ± 22.35	0.484
eGFR n (%)				
≥ 90	4 (25.0)	12 (75.0)	16 (18.6)	
60-89	6 (15.0)	34 (85.0)	40 (46.5)	
30-59	8 (27.6)	21 (72.4)	29 (33.7)	
15-29	0 (0.0)	1 (100)	1 (1.2)	
Stages of CKD n (%)				0.481
1	0 (0.0)	0 (0.0)	0 (0.0)	
2	3 (33.3)	6 (66.7)	9 (10.5)	
3	4 (30.8)	9 (69.2)	13 (15.1)	
4	0 (0.0)	2 (100)	2 (2.3)	
Total CKD, n (%)				
(Stages 1-4)	7 (29.2)	17 (70.8)	24 (27.9)	

Table 4.2 shows the *biochemical characteristics, estimated glomerular filtration rate (eGFR) and stages of Chronic Kidney Disease among Hypertensives stratified by gender*. High ACR= \geq 2.5 and 3.5 for males and females respectively. CKD: Chronic Kidney Disease; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; FGF23: Fibroblast growth factor-23; UACR: Urine Albumin creatinine ratio; e GFR: Estimated glomerular filtration rate, n:number of respondents, p-value <0.05: for statistical significance

Table 4.3 shows that more angiotensin converting enzyme inhibitors were used by the respondents while calcium channel blockers were less used. Again, there were significant differences in the systolic ($P=0.003$) and diastolic ($P=0.006$) blood pressure between subjects with only hypertension and those with CKD. Biochemical analytes (urea, creatinine, phosphate, calcium, and eGFR) were significant between respondents with hypertension and CKD except sodium ($P=0.819$). The mean plasma concentration of FGF23 in respondents with hypertension was 4153 ± 2174.27 and 1434.76 ± 266.86 for respondents with CKD.

4.4: Biochemical characteristics of respondents stratified by disease state (hypertension, normotension, and Chronic Kidney Disease)

Figure 4.1 (a-d) compares the plasma levels of FGF23, calcium, sodium and phosphate concentrations among the study subjects. Biochemical characteristics of respondents were stratified by disease state (hypertension, normotension, and Chronic Kidney Disease). There were no significant differences in the plasma concentration of sodium between hypertension and CKD ($P=0.808$) as depicted by Figure 4(c). There was however significant difference in the sodium concentration between respondents with hypertension and those without hypertension ($P=0.012$), and those without hypertension (normotension) and CKD ($P=0.05$) as shown in Figure 4 (c). Again, there were no significant differences in the concentration of phosphate between respondents with hypertension and those without hypertension but differed significantly in respondents with hypertension and CKD as in Figure (4.1b). There was however significant difference in the plasma concentrations of FGF23 and calcium among normotensive, hypertensive and CKD subjects (4.a and 4.d).

Table 4.3: Demographic and biochemical characteristics among Hypertensives and Hypertensives with CKD

Variable	HTP (n = 62)	HTP/CKD (n = 24)	P-value
Age group(years) n (%)			0.415
<40	1 (1.6)	2 (8.3)	
40-49	7 (11.3)	2 (8.3)	
50-59	17 (27.4)	10 (41.7)	
60-69	15 (24.2)	5 (20.8)	
70-79	16 (25.8)	3 (12.5)	
≥ 80	6 (9.7)	2 (8.3)	
Duration of HTP(years)			0.500
1-5.	26 (41.9)	10 (41.7)	
6-10.	24 (38.7)	10 (41.7)	
11-15.	7 (11.3)	4 (16.7)	
16-20.	5 (8.1)	0 (0.0)	
Drug of choice for HPT			0.780
ACEI	48 (77.4)	18 (75.0)	
CaCB	2 (3.2)	0 (0.0)	
β2-Blockers	5 (8.1)	2 (8.3)	
ACEI + CaCB	5 (8.1)	2 (8.3)	
ACEI + β2-Blockers	2 (3.2)	2 (8.3)	
Weight (Kg)	62.19 ± 13.74	67.71 ± 14.46	0.103
Blood pressure (mmHg)			
SBP	140.39 ± 17.29	155.88 ± 28.15	0.003
DBP	81.10 ± 6.82	87.75 ± 15.18	0.006
Phosphate (mmol/L)	1.85 ± 1.12	6.73 ± 3.02	<0.0001
Sodium (mmo/L)	149.57 ± 13.27	148.85 ± 12.53	0.819
Calcium (mmol/L)	7.64 ± 2.08	3.95 ± 1.35	<0.0001
FGF23 (pg/mL)	1434.76 ± 266.86	4153 ± 2174.27	<0.0001
Urea (mmol/L)	5.63 ± 4.45	15.74 ± 8.45	<0.0001
Creatinine (μmol/L)	89.18 ± 19.76	131.39 ± 44.92	<0.0001
eGFR, mL/min/1.73 m²	76.07 ± 21.01	54.00 ± 17.54	<0.0001

Table 4.3 shows the demographic and biochemical characteristics among Hypertensives and Hypertensives with CKD: ACEI: Angiotensin converting enzyme inhibitor; CaCB: calcium channel blocker; β2: beta. A P value <0.05 is considered statistically significant

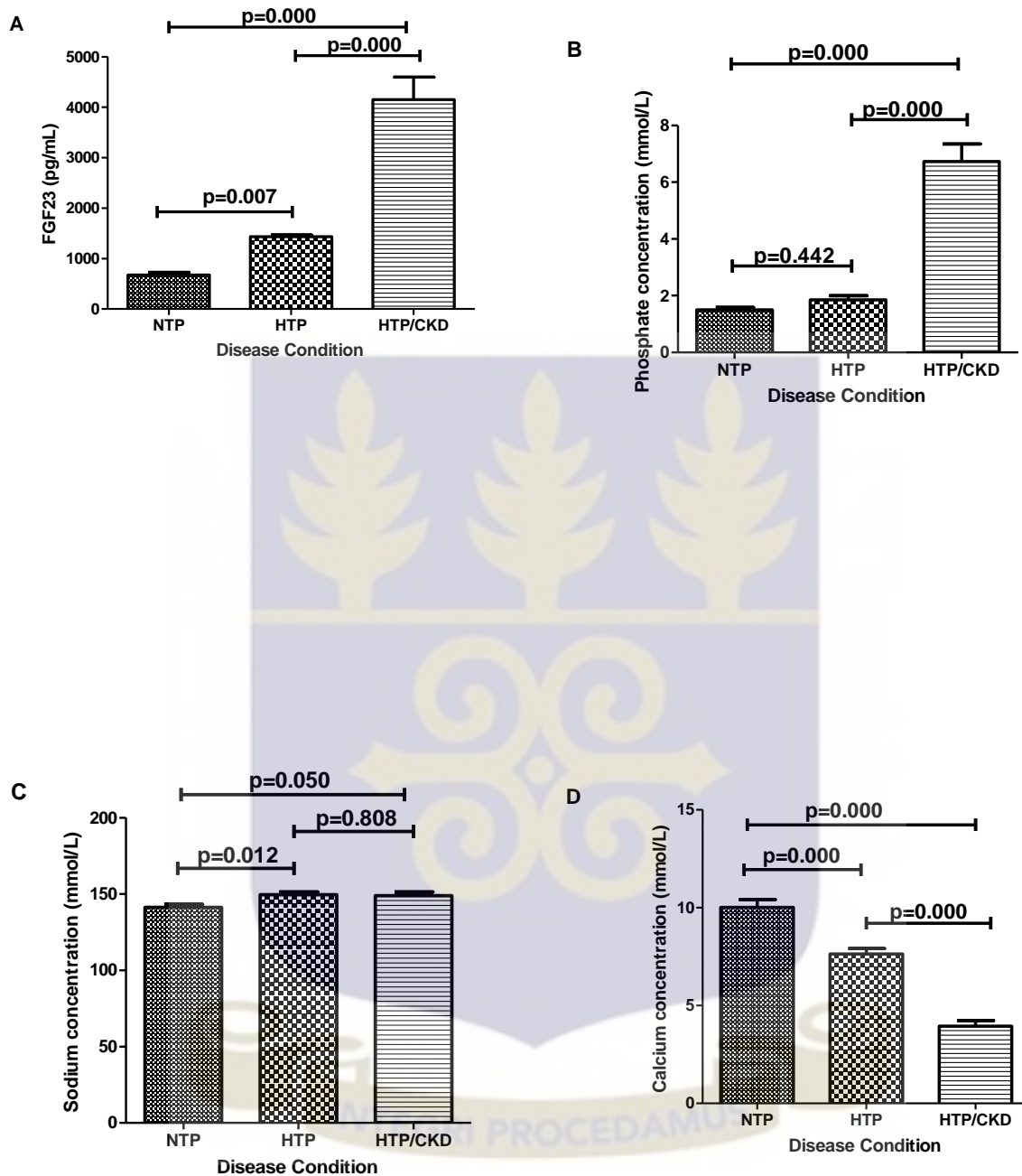


Figure 4.a-d depicts a comparison of the Plasma concentrations of FGF23 (a), Phosphate (b), Sodium(c) and Calcium (d) among Normotensives, Hypertensives and Hypertensive with CKD. NTP: Normotension (high blood pressure), HTP: hypertension, HTP/CKD: subjects with both hypertension and CKD. P-value: statistical significance at 0.05 or less. Arrow bar (\bar{I}): showing statistical difference between or among subjects

4.5: Relationship between FGF23, anthropometric and demographic characteristics among Hypertensives (Upper portion) and Hypertensives with CKD (Lower portion)

Multivariate analysis of biochemical and demographic characteristics is depicted in Table 4.4. The upper part of Table 4.4 represented respondents with hypertension without CKD and the lower portion represented respondents with CKD. There was an inverse correlation between FGF23 and age, sodium, calcium and estimated GFR. However, FGF23 had positive correlation with weight, systolic and diastolic pressure, urea, phosphate, and creatinine concentrations. Increased FGF23 correlated directly with an increase in blood pressure as shown the upper part of Table 4.4. A positive and direct correlation also existed between blood pressure and estimated GFR. The lower portion that represented respondents with CKD showed that age ($R = -0.004$), calcium ($R = -0.504$) and estimated GFR ($R = -0.688$) inversely correlated with the plasma concentration of FGF23, all decreasing when plasma FGF23 increases. There was however a positive correlation between FGF23 and sodium, urea, creatinine, phosphate and blood pressure (diastolic and diastolic pressure). Though sodium negatively correlated with FGF23 in respondents with hypertension, it however correlated positively with FGF23 in respondents with hypertension associated CKD ($R = 0.05$). There was significant differences in the plasma concentration of FGF23 with calcium, urea, creatinine, and phosphate in respondents with only hypertension ($p < 0.005$) and those with CKD. There was however no significant difference in the concentrations of sodium, phosphate and FGF23 in respondents with CKD.

Table 4.4: Relationship between FGF23, anthropometric and demographic characteristics among Hypertensives (Upper portion) and Hypertensives with CKD (Lower portion)

Variables		FGF23	Age	Weight	SBP	DBP	Phos	Na	Ca	Pur	Pcr	eGFR
FGF23	R	1	-0.133	0.13	.396**	.377**	.679**	-0.008	-.568**	.700**	.715**	-.505**
	P-value		0.221	0.233	0.000	0.000	0.000	0.940	0.000	0.000	0.000	0.000
Age	R	-0.004	1	-.462**	-0.189	-0.211	-0.074	.239*	0.161	-0.174	-0.015	-.235*
	P-value	0.985		0	0.081	0.051	0.497	0.026	0.139	0.11	0.892	0.03
Weight	R	0.016	-0.362	1	0.141	.240*	0.142	-0.113	-0.204	.281**	.225*	0.056
	P-value	0.939	0.082		0.195	0.026	0.191	0.302	0.059	0.009	0.037	0.606
SBP	R	0.354	-0.091	0.154	1	.699**	.258*	0.016	-.320**	.261*	.312**	-0.175
	P-value	0.089	0.674	0.473		0	0.017	0.887	0.003	0.015	0.003	0.108
DBP	R	0.316	-0.311	.408*	.845**	1	0.184	-0.009	-.245*	.323**	.311**	-0.134
	P-value	0.132	0.139	0.048	0		0.089	0.933	0.023	0.002	0.004	0.219
Phos	R	0.341	0.1	-0.099	0.032	-0.119	1	0.055	-.524**	.634**	.475**	-.408**
	P-value	0.103	0.642	0.647	0.882	0.579		0.617	0.000	0.000	0.000	0.000
Na	R	0.059	.414*	0.151	-0.13	-0.151	0.282	1	-0.058	-0.05	0.086	-0.066
	P-value	0.785	0.044	0.48	0.546	0.482	0.182		0.597	0.65	0.432	0.548
Ca	R	-.504*	0.16	-0.253	-0.336	-0.312	-0.066	0.039	1	-.499**	-.420**	.310**
	P-value	0.012	0.455	0.232	0.109	0.138	0.759	0.858		0	0	0.004
Pur	R	.627**	0.032	0.346	0.204	0.212	.487*	.458*	-0.353	1	.601**	-.386**
	P-value	0.001	0.881	0.098	0.338	0.321	0.016	0.024	0.09		0	0.000
Pcr	R	.704**	-0.121	.519**	0.365	0.368	0.163	0.256	-.455*	.699**	1	-.829**
	P-value	0	0.572	0.009	0.08	0.077	0.445	0.227	0.026	0.000		0.000
Egfr	R	-.688**	-0.033	-0.297	-0.232	-0.144	-0.236	-.443*	.420*	-.664**	-.889**	1
	P-value	0	0.879	0.158	0.275	0.502	0.267	0.03	0.041	0	0	

Table 4.4 depicts the relationship between FGF23, anthropometric and demographic characteristics among Hypertensives and Hypertensives with CKD . Ca: calcium, Na: sodium, SBP: systolic blood pressure, DBP: diastolic blood pressure, Phos: phosphate, Pur: plasma urea, Pcr: plasma creatinine, R: Pearson correlation coefficient

4.6: Risk determinants for increase in FGF23 hormone in Hypertensives

The determining risk factors associated with increasing FGF23 plasma concentration as illustrated in Table 4.5 were a duration of hypertension for at least 6 years (OR=1.08, 95% CI:0.38-3.31) and weight (OR=1.02, CI:0.99-1.06) though the association was not significant ($P>0.05$). Hypertension of 1-5 years duration was not a risk factor for increasing FGF23 (OR=1). Again, gender and ageing (>40yrs) did not have a risk effect on FGF23 levels (OR<1). Among the antihypertensive drugs, angiotensin converting enzyme inhibitors (ACEI) was a significant risk factor that can elevate plasma FGF23 levels (OR= 9.1×10^7 ; 95% CI: 1.1×10^6 - 6.9×10^7 , $P<0.0001$). Again, beta-2 blockers and ACEI with calcium channel blockers also posed significant risk (OR>1, $P<0.0001$). However, calcium channel blockers alone, and the combined effect of ACEI with beta-2 blockers were not risk factors that can increase FGF23 levels in individuals on such medications. Systolic and diastolic blood pressures were also identified as risk dominants of FGF23 levels in plasma though the risk of association was not significant. Furthermore, phosphate (OR=4.67, 95%CI: 1.32-5.40) was identified as a significant ($P=0.006$) biochemical risk factor associate with increase in plasma levels of FGF23. Other biochemical risk factors included urea (OR=1.09, CI: 0.87-1.36), Creatinine (OR=1.07; CI: 0.94-1.23), and estimated GFR (OR=1.04, CI: 0.89-1.21) though the risk of association between the plasma levels of FGF23 and urea, creatinine and estimated GFR was not significant ($P>0.05$).

4.7: Biochemical and demographic characteristics of respondents with CKD

Again, Table 4.6 depicts the biochemical characteristics of respondents with stages 2, 3 and 4 of CKD. There was no significant difference in the age and blood pressure (systolic and diastolic pressure) in all the stages of CKD. Similarly, the duration of hypertension and the choice or type

of antihypertensive drug used in managing respondents with hypertension did not also show any significant difference in all the stages of CKD. However, there was significant difference in weight of respondents with stages 2 and 4 of CKD ($P=0.001$) but not stage 3. Respondents with CKD stage 4 have the highest mean weight (kg) of 99.00 ± 4.24 . Also, concentrations of calcium and phosphate in all the stages of CKD (1-4) showed no significant difference ($P>0.05$). Sodium and FGF23 concentrations however showed significant differences in concentration in all the stages of CKD ($P<0.05$). Stage 4 CKD had the highest mean concentrations of sodium and FGF23.

4.8: Biochemical characteristics of respondents with various stages or grades of hypertension

The biochemical characteristics of subjects with hypertension shows that, among study participants with hypertension of various stages or grades (1 -3), there was no significant difference in the plasma level of FGF23 between Grades 1 and 2, Grades 2 and 3. There was however significant difference in FGF23 concentration between respondents with Grades 1 and 3 hypertension ($P< 0.05$) as shown in figure 4.2A. The mean concentration of FGF23 was higher in Grade 1 hypertension than all in all the grades and lower in Grade 1 hypertension. Again, there was difference in calcium concentrations among the various grades of hypertension though the difference were insignificant ($P>0.05$) as shown in figure 2D. Plasma concentrations of sodium and phosphate were also different among the various grades of hypertension although the differences were not statistically significant as depicted in figures 2B and 2C. Phosphate concentration was however increased in magnitude of the grades of hypertension (i.e phosphate: Grade 1< 2<3) as in figure 4.2B. Calcium however decreased in concentration from grade 3 hypertension to grade 1 (i.e Ca: 3<2<1) as in figure 2D.

Table 4.5: Risk determinants for increase in FGF23 hormone in Hypertensives

Variable	OR (95% CI)	P-value
Gender		
Male	1	
Female	0.52 (0.18-1.57)	0.247
Age group n (%)		
<40*	1	
40-49	0.14(0.01-2.52)	0.184
50-59	0.29 (0.02-3.67)	0.342
60-69	0.17 (0.01-2.26)	0.178
70-79	0.09 (0.01-1.39)	0.086
≥ 80	0.17 (0.01-2.98)	0.224
Duration of HPT		
1-5	1	
6-10	1.08 (0.38-3.31)	0.880
11-15	1.49 (0.36-3.20)	0.587
16-20	-	-
Drug of choice for HPT		
ACEI	9.1 x10 ⁷ (1.1 x10 ⁶ -6.9 x 10 ⁷)	<0.0001
CaCB	1	
β2-Blockers	9.7 x10 ⁷ (7.5x10 ⁶ -10.5 x10 ⁷)	<0.0001
ACEI + CaCB	9.7 x10 ⁷ (7.5x10 ⁶ -10.5x10 ⁷)	<0.0002
ACEI + β2-Blockers	-	-
Weight (Kg)	1.04 (0.99-1.06)	0.236
Blood pressure (mmHg)		
SBP	1.04 (0.99-1.06)	0.121
DBP	1.05 (0.95-1.09)	0.573
Phosphate (mmol/L)	4.67 (1.32-5.40)	0.006
Sodium (mmo/L)	0.98 (0.87-1.10)	0.693
Calcium (mmol/L)	0.47 (0.23-0.96)	0.037
Urea (mmol/L)	1.09 (0.87-1.36)	0.446
Creatinine (μmol/L)	1.07 (0.94-1.23)	0.285
eGFR, mL/min/1.73 m²	1.04 (0.89-1.21)	0.639

Table 4.5 shows risk factors associated with increasing in FGF23 hormone in Hypertensives
OR: Odd ratio; ACEI; angiotensin converting enzyme inhibitors; CaCB:Calcium channel blockers; e GFR: estimated glomerular filtration rate; SBP:systolic blood pressure; DBP: diastolic blood pressure. An OR> is considered a risk factor, a P value < 0.05 shows significant risk.

Table 4.6: Biochemical and demographic characteristics of respondents with CKD

Variable	CKD STAGES			P-value
	CKD 2	CKD 3	CKD 4	
Age group n (%)				0.415
<40	2 (22.2)	0 (0.0)	0 (0.0)	
40-49	0 (0.0)	2 (15.4)	0 (0.0)	
50-59	4 (44.4)	4 (30.8)	2 (100)	
60-69	2 (22.2)	3 (23.1)	0 (0.0)	
70-79	0 (0.0)	3 (23.1)	0 (0.0)	
≥ 80	1 (11.1)	1 (7.7)	0 (0.0)	
Duration of HPT				0.643
1-5.	4 (44.4)	6 (46.2)	0 (0.0)	
6-10.	4 (44.4)	5 (38.5)	1 (50.0)	
11-15.	1 (11.1)	2 (15.4)	1 (50.0)	
16-20.				
Drug of choice for HPT				0.629
ACEI	8 (88.9)	8 (61.5)	2 (100)	
β2-Blockers	0 (0.0)	2 (15.4)	0 (0.0)	
ACEI + CaCB	0 (0.0)	2 (15.4)	0 (0.0)	
ACEI + β2-Blockers	1 (11.1)	1 (7.7)	0 (0.0)	
Weight (Kg)	67.22 ± 8.84*	63.23 ± 12.73	99.00 ± 4.24*	0.001
Blood pressure (mmHg)				
SBP	150.44 ± 34.26	156.85 ± 23.71	174.00 ± 33.94	0.575
DBP	88.00 ± 15.21	85.31 ± 14.80	102.50 ± 17.68	0.343
Phosphate (mmol/L)	5.72 ± 1.51	7.51 ± 3.80	6.18 ± 0.39	0.394
Sodium (mmo/L)	140.68 ± 12.13*	152.81 ± 9.70*	159.90 ± 15.98*	0.027
Calcium (mmol/L)	4.29 ± 0.67	3.75 ± 1.66	3.69 ± 0.06	0.644
FGF23 (pg/mL)	2796.56 ± 254.91*	4871.62 ± 2406.18*	5586.50 ± 3369.63	0.048

Table 4.6 shows the biochemical and demographic characteristics of respondents with various stages of CKD *significantly different from CKD 2, +significantly different from CKD 3 at $p < 0.05$ HPT: hypertension

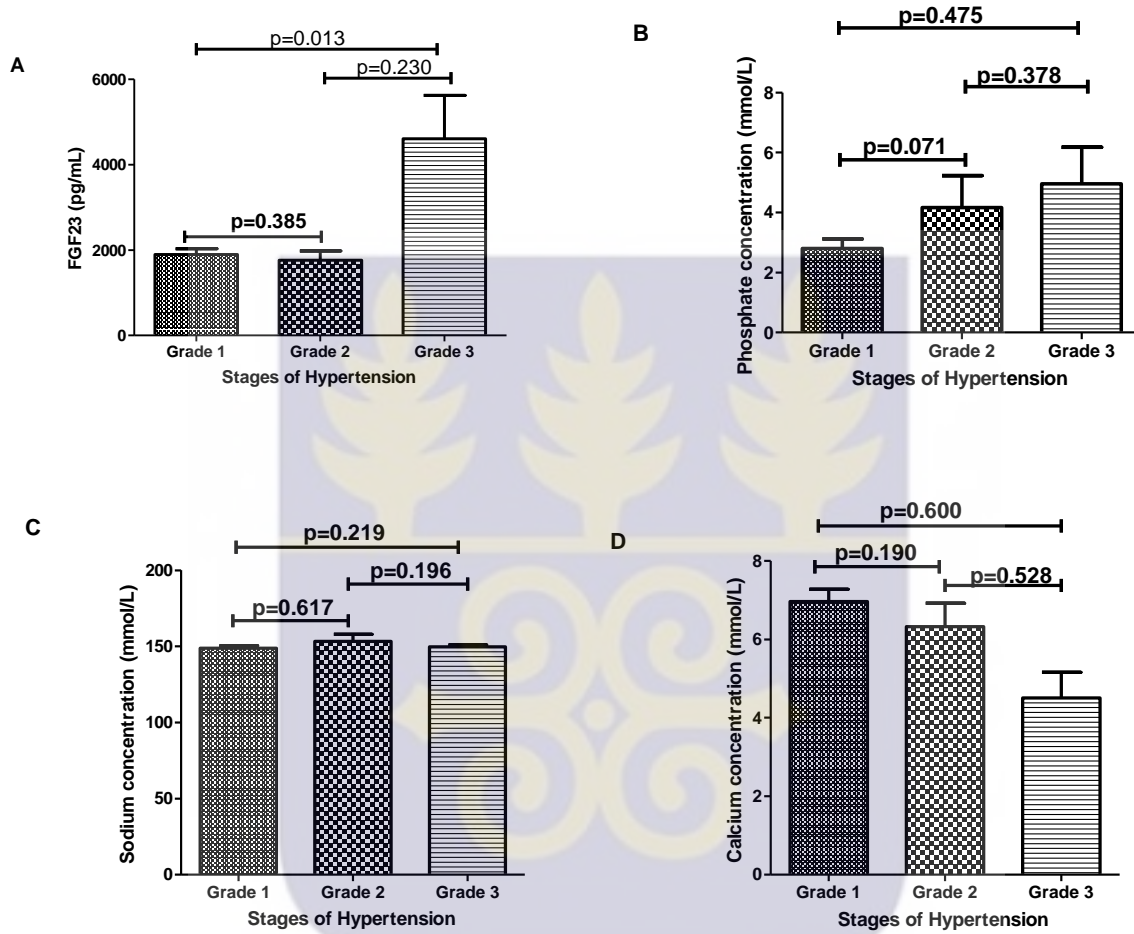


Figure 4.2a-c shows a comparison of the plasma concentrations of FGF23, Phosphate, Sodium and Calcium among various grades or stages of Hypertensives. $p < 0.05$ is considered significant difference.

CHAPTER FIVE

5.1 Discussion

The study was a cross-sectional study in which study subjects were selected by convenience sampling. Outpatients with hypertension for at least a year and on antihypertensive drugs were selected as well as blood donors with no history of hypertension as controls. The study compared the biochemical characteristics among respondents. The plasma level of FGF23 among the study participants was measured and compared among them. The study also determined the association of biochemical and anthropometric characteristics and the level of FGF23. Risk anthropometric and demographic factors associated with high level of FGF23 were also assessed. In this study, one hundred and forty six (146) individuals were involved. Eighty-six (86) respondents were hypertensives on regular antihypertensive management whiles sixty (60) were healthy blood donors with no history of hypertension. Approximately, 79% of all hypertensive outpatients were females. In screening all subjects for CKD, the study showed that 27.9% of all hypertensives had CKD defined by the elevated concentrations of urea, creatinine, microalbuminuria and estimated glomerular filtration rate (Table 4.2).

In assessing the biochemical characteristics of the subjects with, and without hypertension, the analytical findings showed a significant difference in the concentrations of urea, calcium, sodium, phosphate, urine creatinine albumin ratio, and estimated glomerular filtration rate. The study showed that the plasma level of FGF23 in hypertensive subjects was significantly higher than in those without hypertension (Table 4.1). Again, study participants with CKD have higher plasma level of FGF23 than those with no CKD (Table 4.3). These findings could be due to differences in functional renal reserve. The significant difference in FGF23 plasma levels

between hypertensives & normotensives observed in this study agrees with the results of a study in health professionals (Gutierrez *et al.*, 2011). In that study, participants with established hypertension had higher FGF23 concentrations than participants who did not have hypertension (Gutierrez *et al.*, 2011). Gutierrez *et al.* (2011) also established that history of hypertension caused significant increase in the plasma concentration of FGF23 unlike in normotensives with functional renal capacity. Similarly, Mirza *et al.* (2011) and Marsell *et al.* (2009) are all in agreement with the finding of this study that hypertension was associated with high FGF23 levels than normotensives (Figure 4a).

According to the National institute of health, CKD is a worldwide public health disaster that affects millions of people; various studies conducted by research scientists have reported hypertension as one of the leading and common cause of CKD. In this study, 27.9% of the study population with hypertension had CKD in the Techiman municipality as compared to 46.9% of CKD in outpatients with hypertension in Greater Accra (Osafo *et al.*, 2011). In another study, 22% of the study population with hypertension in South-Western Ghana had CKD (Ephraim *et al.*, 2015). The difference in the findings of CKD in hypertensive outpatients may be attributed to the use of the CKD-EPI in this study as compared to the MDRD equation for GFR estimation used in the studies. The MDRD equation is known to over estimate the presence of CKD compared with the CKD-EPI (Kitiyakara *et al.*, 2012). Sample size variation is also a likely factor that accounted for the different findings. Furthermore, 72.1% of the study population with hypertension had low microalbuminuria (<2.5 mg/mol for males and <3.5 mg/mol for females) as defined by UACR. Some patients with the low microalbuminuria will eventually progress to high microalbuminuria state and hence CKD. Regular monitoring and management of their hypertensive condition is therefore necessary to avert CKD development. Based on the kidney

Disease Outcomes Quality initiative (K/DOQI) of the National Kidney Foundation, out of the 27.9% study population with CKD, CKD- 2, CKD-3, and CKD-4 stages were found. Chronic Kidney Disease was higher in the female population (70.8%) than in the male population (29.2%) as observed in this study. This may be due to the large female than male respondents in the study.

Phosphate, especially intracellular phosphate is recognized to play a major physiologic role in the regulation of FGF23 (Gutierrez *et al.*, 2009). In the florescence of calcium, phosphate plays a major role in the pathogenesis of vascular calcification in CKD patients (Giachelli *et al.*, 2005). Again, hyperphosphatemia in ESRD causes soft tissue calcification (Gutierrez *et al.*, 2009). Some researchers have observed that the adverse role of hyperphosphatemia is not limited to only patients with CKD as it correlated significantly with the severity of coronary artery disease, coronary artery stenoses and occlusion (Kestenbaum *et al.*, 2005). Also, severe plasma phosphate level was associated with adverse cardiovascular outcomes (Tonelli *et al.*, 2005). Against the background of hyperphosphatemic effects in CKD patients, it was found that phosphate concentrations showed significant difference among normotensives, hypertensives, and hypertensive with CKD patients. The mean plasma levels of phosphate was within normal levels in study subjects without hypertension (normotensive subjects) unlike in hypertension and in CKD. Phosphate level was high in hypertensive subjects than in normotensive subjects and higher in CKD than in both normotensive and hypertensive subjects (Figures 4a-d). The normal mean phosphate levels phosphate concentration in the normotensive participants therefore makes the occurrence of cardiovascular and other effects of hyperphosphatemia unlikely as reported in previous studies (Tonelli *et al.*, 2005). Tonelli and colleagues reported that phosphate levels in non-renal hypertensive individuals were lower than in renal and hypertensive individuals.

This was attributed to the fact that renal damage as a result of hypertension could have resulted in impaired phosphate excretion via the kidneys and hence, phosphate retention. Similarly, as found in this study, Danilo *et al* (2007) reported significant difference in the phosphate levels between renal and non-renal patients. They observed that phosphate levels in patients with advanced kidney failure were significantly higher than in those without kidney failure

Fibroblast Growth Factor-23 is recently identified as a new and major regulator of phosphate balance especially in patients with CKD (Juppner, 2011; Danilo *et al.*, 2007). Excess activity of FGF23 have been found in clinical studies to cause hypophosphatemia, low $1, 25\text{-OH}_2\text{D}_3$ levels, and osteomalacia (Jonsson *et al.*, 2004). Again, experimental studies have demonstrated that overexpression of FGF23 gene *in vivo* deranged calcium-phosphate metabolism whereas inactivation of the FGF23 gene lead to hyperphosphataemia and high circulating $1, 25\text{-OH}_2\text{D}_3$ levels (Shimada *et al.*, 2001, 2004). As a result of dietary phosphate load, FGF23 is secreted and the increase in FGF23 levels in response to dietary phosphate load triggers renal loss of phosphate (Shimada *et al.*, 2001). However, in progressive CKD, FGF23 levels increase in magnitude with the decline of renal function, thereby increasing serum phosphate concentration (Panichi *et al.*, 2001; Larsson *et al.*, 2003, Gutierrez *et al.*, 2005). In this study, the plasma level of FGF23 in hypertensive patients was significantly higher than in those without hypertension. It was observed that the level of FGF23 was three times higher in hypertensive than in normotensives. This is consistent with those of prior studies (Gutierrez *et al.*, 2011; Marsell *et al.*, 2009; Mirza *et al.*, 2011). Though the exact cause of this difference in plasma level of FGF23 was not established, it is thought that the higher phosphate concentrations in hypertensive patients than in normotensives may be attributed to this higher plasma FGF23 in hypertensives

than in normotensives. This seems to agree with the suggestion that hyperphosphatemia stimulates FGF23 secretion (Ferrari *et al.*, 2005).

Another key finding of this study was the significant difference in FGF23 plasma level between patients with hypertension and those with CKD. The mean plasma concentration of FGF23 was about three times the plasma concentration in hypertension. This significant difference could be as a result of a decrease in renal function as similarly observed by Danilo and colleagues in the mild to moderate CKD study. Again, Gutierrez *et al* (2009) reported similar increase in the level of FGF23 in early stages of CKD. It was also found in this study that plasma level of FGF23 was highest in CKD population, followed by hypertensive and lowest in the normotensive population (Figure 4a). This may again be attributed to the differences in functional renal capacity of the normotensives than the hypertensives and CKD. The higher FGF23 concentrations in hypertensives than in normotensives could also be due to the sodium conserving effect of FGF23 (Olena *et al.*, 2013) that triggers a feedback stimulation of FGF23 secretion. Olena *et al* (2013) demonstrated in experimental studies that mice with high FGF23 have high sodium in their blood and a high systolic and diastolic blood pressure. Gutierrez *et al* (2009) reported that the history of hypertension was associated with elevated FGF23 levels. This means that FGF23 could also increase in patients with hypertension as it was found in this study in which the mean level of FGF23 was significantly higher in individuals with hypertension than individuals without hypertension.

Again, as reported in several studies, it was found in this study that FGF23 plasma concentration was increased significantly from one stage of CKD to the other stage (CKD2-4) as shown in Table 4.6. The FGF23 concentration is increased in magnitude as CKD progresses (i.e FGF23:CKD2<CKD3<CKD4). Again, diminished renal function as CKD deteriorates may

account for the significant increase in FGF23 levels across the various stages of CKD. Gutierrez *et al* (2009) reported similar increase in FGF23 in CKD, reaching 2-5 times the upper limit during the early and intermediate stages of CKD. It was also reported that FGF23 level can reach 200 times or more of the normal level in advanced or end stage renal disease (Gutierrez *et al.*, 2009). The progressive increase in FGF23 parallel to CKD stages therefore makes FGF23 a novel marker for predicting CKD progression. In similar studies, Danilo and colleagues (2007) reported that FGF23 is increased in magnitude as CKD progresses from stages 2 to 5. This is also consistent with the findings of other studies (Larsson *et al.*, 2003; Isakova *et al.*, 2011) although the exact stage of CKD when FGF23 levels first became significantly elevated was different across studies.

In a multivariate logistic analysis, antihypertensive drugs were identified to be associated with increasing plasma level of FGF23 although the degree of association was not significant with some of the drugs in individuals without CKD. Angiotensin converting enzymes inhibitors (ACEI), beta-2 blockers, and a combination of ACEI and calcium channel blockers were all found to be significantly associated with increasing plasma FGF23 levels. There was significant and strong association of ACEI with the plasma concentration of FGF23 (OR=9.1 X 10⁶, 95% CI: 1.1 X10⁶-6.9 X10⁷, $P<0.0001$), thereby making ACEI a significant risk factor among all other antihypertensive medications. However, calcium channel blockers and beta-2 blockers in combination with ACEI had no significant association with increasing the plasma concentration of FGF23 although they were identified as risk factors that can increase FGF23 in plasma. This is consistent with a study in which antihypertensive drugs especially angiotensin converting enzyme inhibitors was identified to be associated with increased FGF23 in patients on antihypertensive treatment who progressed to renal end point than those who had no renal

damage (Danilo *et al.*, 2007). Studies will therefore be needed to ascertain the predictive ability of antihypertensive drugs for FGF23 progression. Furthermore, the results of this study also confirm those of prior studies that examined association of increased FGF23 and CKD markers, sodium, age, blood pressure, calcium and phosphate in both CKD and non-CKD populations (Danilo *et al.*, 2007). In this study, ageing and gender were not risk factors associated with increasing plasma level of FGF23 in both hypertensives with and without CKD. This however, contrasts Danilo and colleagues (2007) who reported ageing to be significantly associated with increase in FGF23 levels. According to these researchers, every 5-year increase in age was associated significantly with 2.1 RU/ml higher FGF23 ($P < 0.001$). Again, urea, and creatinine were all associated risk factors with increasing in FGF23 levels. These findings are consistent with other authors of similar studies. According to Danilo and colleagues (2007), each 0.1mg/dl increase in creatinine was associated with 3.4RU/ml higher FGF23 ($P < 0.001$). However, the association between urea and FGF23 levels is yet unknown though it is believed to be associated with an increase in FGF23 levels as established by this study. It was also found that phosphate was significantly associated with increasing FGF23 levels, thereby showing that phosphate load is a major risk factor for elevating FGF23 levels. This is in line with other research findings. In one of such studies, each 500mg increase in daily phosphorus intake was associated significantly with 3.4 RU/ml higher FGF23 (Danilo *et al.*, 2007). This finding however contrasts with another study that found no association between dietary phosphate and FGF23 (Isakova *et al.*, 2011).

Furthermore, there was a significant correlation between serum phosphate and calcium, serum urea, serum creatinine and estimated GFR in hypertensive subjects without CKD (Table 4.4). A significant inverse correlation between phosphate and calcium, and estimated GFR was found. Phosphate also correlated significantly with urea, creatinine and FGF23. This suggests that

hypertensive patients, though did not have established CKD, may have diminished kidney function indicated by the low GFR resulting in the plasma accumulation of urea, phosphate and creatinine. Again the correlation between phosphate concentration and calcium concentration could be due to minor derangements of calcium-phosphate metabolism even in non-renal patients. These findings agreed with that of Tonelli and colleagues. These findings agreed with Tonelli *et al* (2005) who reported that low GFR was associated with high serum phosphate concentrations. Changes in calcium-phosphate metabolism could pose adverse hyperphosphatemic effects or complications in both CKD and non-CKD patients as observed by Danilo and colleagues in the mild to moderate kidney Disease (MMKD) study (2007). The correlation between the GFR and phosphate in the non-CKD patients could be attributed to minimal kidney damage that caused phosphate retention. In non-renal diseased patients, Gutierrez *et al* (2011) and Danilo *et al* (2007) also reported similar significant correlation between FGF23 and phosphate. Again, systolic and diastolic blood pressure significantly correlated with FGF23 in individuals with hypertension. Though there is no comparable data available on this finding, it is thought that the significant association between history of hypertension and FGF23 levels (Gutierrez *et al.*, 2011) could be the result of this significant positive correlation.

Similarly, FGF23 levels in CKD individuals showed a significant inverse correlation with estimated GFR, sodium and calcium. This is consistent with the findings in other studies. In such studies, increased FGF23 levels was found to correlate significantly with decreased estimated GFR (Danilo *et al.*, 2007; Gutierrez *et al.*, 2011; Marsell *et al.*, 2009; Parker *et al.*, 2010; Juppner, 2011). In another study, Kelsey and colleagues (2012) found similar significant correlation between elevated FGF23 and decreased estimated GFR. The increased concentration

of phosphate that correlated significantly with high plasma FGF23 levels in both CKD and non-CKD study populations corroborated with other studies (Gutierrez *et al.*, 2011; Danilo *et al.*, 2007). In contrast to these findings, increase in concentrations of FGF23 and phosphate was found not to have any significant correlation in both individuals with CKD or have functional renal reserve (Isakova *et al.*, 2011). The different findings could possibly be that phosphate was strongly associated with functional kidney reserve than in patients with CKD. Differences in dietary phosphate intake among respondents may have played a role in the contrasting findings. Robust population-based studies to include individuals across a broad spectrum of kidney function and socioeconomic status may be needed to ascertain these possibilities.

Finally, FGF23 was found to be elevated in hypertensive respondents. The increase in the plasma level of FGF23 was higher in Grade 1 hypertension than in Grade 2 but highest in 3 than in both grades and stages of hypertension. This could be due to differences in antihypertensive management approach of patients and the type of antihypertensive medication as this study have found some antihypertensive drugs to significantly increase FGF23 levels. Though no comparable data or study is available to ascertain this finding, it is the view of the researcher that those hypertensives with elevated FGF23 and in CKD stage 3 may have a degree of renal damage that may develop into CKD. Differences in dietary phosphate load by the individual hypertensive subjects may also accounted for this. Prudent and regular clinical monitoring may therefore be needed to avert developing CKD.

CHAPTER SIX

6.0 CONCLUSION, LIMITATIONS AND RECOMMENDATIONS

6.1 Conclusion

The plasma level of FGF23 in patients with CKD was significantly higher than those with hypertension and those without hypertension (normotensives). Among outpatients with hypertension, 27.9% had CKD, ranging from CKD stages 2 to 4. Plasma level of FGF23 increased significantly in magnitude with the progression of CKD from stages 2 to stages 4. There was also a significant association between FGF23 and angiotensin converting enzyme inhibitors, β 2-blockers as well as a combination of angiotensin converting enzymes with calcium channel blockers. Finally, higher levels of FGF23 correlated inversely with decreased estimated GFR, age, and sodium but positively correlated with serum urea, creatinine, phosphate and blood pressure in both CKD and non-CKD populations. The association of systolic and diastolic blood pressure with FGF23 could serve as a biomarker for progression of hypertension and CKD. In effect, FGF23 could serve as a biomarker for monitoring the prognosis and management of patients with hypertension and CKD and to help improve their clinical outcome.

6.2 Limitations

Among the limitations of this study, the sample size was small compared with other studies. The proposed 309 expected to be used for this study was reduced to 146 due to the high cost of FGF23 ELISA KIT. Again, kidney function was assessed based on single measurement of urea and creatinine as well as estimated GFR and estimated urine albumin-creatinine ratio. The CKD-EPI equation which is not validated in the Ghanaian population was used to estimate GFR and

this may over or underestimated the GFR. Finally, iron, parathyroid hormone and 1,25-dihydroxyvitamin D which are found to be associated with elevated FGF23 were not also assessed to affirm those found in this study to have associated with elevated FGF23. However, the association of elevated systolic and diastolic pressure and elevated FGF23 could serve as a biomarker for hypertension and hypertension associated CKD.

6.3 Recommendations

On the basis of these findings, dietary intake of phosphate by hypertensives and CKD patients need to be controlled. Factors such as blood pressure and antihypertensive drugs need to also be monitored from time to time. Further studies are therefore needed to assess and ascertain these factors. Since hyperphosphatemia stimulates FGF23 secretion which has serious adverse complications such as left ventricular hypertrophy, vascular calcification and death, it is necessary to include phosphate binders in the treatment of patients with hypertension and CKD to minimize the spurious effects of phosphate toxicity. Finally, the association of blood pressure with elevated FGF23 levels in hypertensive patients needs to further be investigated to ascertain the potential link between FGF23 and blood pressure as blood pressure was found to predict increase in FGF23 levels.



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APPENDICES

Appendix I: Ethical issues

Consent Form:

Title: Determination of Plasma level of Fibroblast Growth Factor 23 in Ghanaian

Individuals with Hypertension and Chronic Kidney Disease

Dear Volunteer,

This form contains information about the research to be conducted. I will like to seek your permission to include you in the study taking place at the Wa Regional Hospital where you are seeking medical care. It is designed to help you understand what the study is about and to be able to make an informed decision whether or not to take part in the study. As part of the study, I will be asking you some questions for which you are at liberty to answer. You will also be asked to sign it (or make your mark in the presence of a witness) after which a copy of the form will be given to you when you accepted to voluntarily take part in the study after you have read it or it has been read and interpreted to your understanding. You are free to ask me any question for clarification if there is anything you do not understand in the form. Your decision not to take part in this study will not in any way affect you and how you are managed in this facility. Thank you for your kind attention.

Why this study

Increasing levels of FGF23 increases the chances of developing hypertension. Poorly controlled hypertension often results in chronic kidney damage. Studies have shown that abnormally high

level of FGF23 is independently associated with CKD mortalities, left ventricular hypertrophy, vascular dysfunction and calcification, uncontrolled hypertension and cardiovascular diseases. This study therefore seeks to provide data on the level of FGF23 in hypertensive patients and the various stages of hypertension in comparison to normotensives and CKD patients that can serve as a biomarker of hypertension and CKD, and the need for its inclusion as a clinical test for hypertension and a guide to the management of nutritional balance in hypertension patients without evidence of CKD.

Procedures

If you agree to take part in this study, I will be asking you some few questions. Your personal demographic data such as your age, sex etc and drug or dietary history will be recorded. Your venous blood and urine samples will be taken for the purposes of the study. Minimal invasive procedure using a sterile syringe and needle will gently be applied on your hand to take the blood sample.

Possible Benefits

There are no direct benefits such as money to you as an individual. However, your participation may help us determine the levels of fibroblast growth factor 23 (FGF23) in hypertensive patients who are at risk of developing chronic kidney disease (CKD). This will help provide a gist as to whether FGF23 can serve as a biomarker or test of hypertension and hypertension associated CKD and helps in management of hypertension.

Possible Risks/Costs

There are no risks/costs associated with this study. The amount of blood collected is harmless, although there may be a slight pain and bruising at the site where the blood will be drawn. You will receive appropriate treatment as necessary. Sterile techniques and disposable, single-use equipment will be used at all times.

Withdrawal from study

Your participation in this study is completely voluntary and if at any point you don't want to continue to be part of the study, you can withdraw immediately and you will not be in any way forced to continue with the study. Your decision to withdraw from the study will definitely not affect how you are treated at the antenatal clinic.

Confidentiality

All information obtained will be treated with strict confidentiality. When results of this study are published in scientific journals or at medical meetings, identities will be kept anonymous. Questionnaires and medical history obtained will also be kept safe in locked cabinets. Soft copies will also be password protected.

Contacts

If you have any question about this study or study-related problems, you may contact me, the principal investigator, **Mr. Biekpe Sylvester (0207180414 or sylvesterbiekpe@gmail.com)** at the **Department of Chemical pathology**, School of Biomedical and Allied Health Sciences, Korle-Bu. You may also contact **Dr. C. Osafo, Department of Medicine and Therapeutics, School of Medicine and Dentistry of the University of Ghana (Tel: 0208867829)** or **Prof. H**

Asare-Anane (0246024002), Head of Department of Chemical Pathology, School of Biomedical and Allied Health Sciences, Korle-Bu, for any further clarification on this study.

You are free to ask any questions. Thank you.

Declaration (Volunteer)

I declare that I have read and understood all the above and hereby agree to voluntarily take part in this study or I declare that the contents of this form have been explained to me and I voluntarily agree to take part in the study.

.....

Participant No:

Name of participant

Date:

.....

Signature or Thumb prints of Participant

Tel:

.....

Name of Witness

Date:

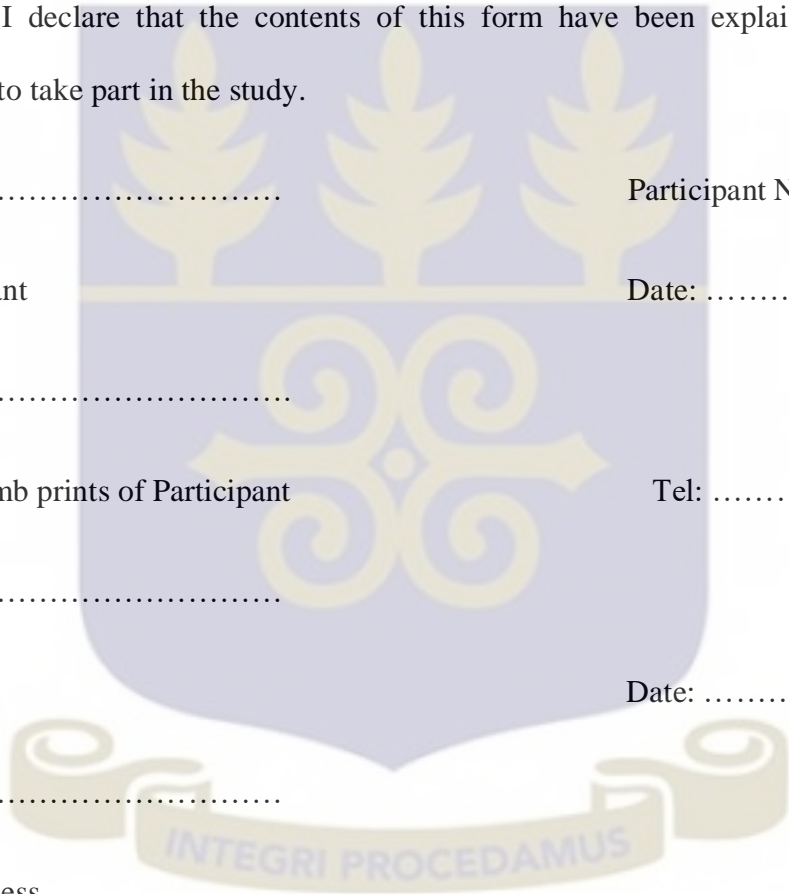
.....

Signature of Witness

.....

Name and signature of Investigator

Date:



Appendix II: Study Abstraction Form

A. Personal Data

Name (Surname in blocks).....

Date Subject code.....

Contact Tel. Number(s).....

E-mail

Postal Address.....

B. Demographics and Anthropometrics

1. Age..... 3. Weight (kg).....

2. Height (m)..... 4. BMI.....

Please tick [] the appropriate box where applicable

7. Sex: [] Male [] Female

C. Medical History

8. Do you have any of the following conditions?

[] Chronic Kidney Disease

[] Hypertension

9. How long has it been since you were diagnosed with the condition(s) in 1 above?

- 1-5 years
- 6-10 years
- 11-15 years
- 16-20 years
- Over 20 years

10. Are you on any treatment for this condition?

- Yes
- No

11. If yes for hypertension, how long have you been on treatment

12. What type of treatment are you on?

- Orthodox/Conventional
- Herbal
- Combined (please specify combination)
- Others (please specify)

13. Can you list the drugs prescribed by your treatment in 4 above (obtained from patients medical folder)

i.

ii.

iii.

D. Social and Recreational Drug Use History

14. Have you ever taken/used any of the following recreational products? Tick as many as apply

Alcohol/Alcoholic Beverage (a)

Cigarettes (b)

Marijuana (c)



APPENDIX III

EQUATIONS

$n = \frac{t^2 x p(1-p)}{m^2}$; where t is the confidence level (CI) at 95%, p is the probability of the general population with hypertension in Ghana, m is the margin of error

GFR= $141 \times \min(\text{Scr}/k, 1)^\alpha \times \max(\text{Scr}/k, 1)^{-1.209} \times 0.993^{\text{Age}} \times 1.159 \times 1.018$ (if female) where;

Scr=standardized serum creatinine in mg/dL, k=0.9 and 0.7 for males and females respectively

α =-0.329 and -0.411 for females and males respectively, min= minimum of Scr/k or 1, max= maximum of Scr/k or 1

UACR: albumin (mg/dl)/creatinine (mmol/dl).

