BIOCHEMICAL CORRELATES OF RENAL DYSFUNCTION AMONG NON-DIABETIC HYPERTENSIVES

A THESIS SUBMITTED IN
FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE
OF

MASTER OF PHILOSOPHY

In the

Department of Molecular Medicine,
School of Medical Sciences

by

MIREKU ERNEST KWASI

KWAME NKRUMAH UNIVERSITY OF SCIENCE & TECHNOLOGY,

KUMASI

SEPTEMBER, 2012

DECLARATION

This is to certify that Ernest Kwasi Mireku undertook this research work which has not been presented for any degree elsewhere under our supervision, and we therefore approve this research work ready for submission.

…… ……..

Ernest Kwasi Mireku Date

(Researcher)

…… ……..

Dr. W.K.B.A. Owiredu. Date

(Supervisor)

…… ……..

Prof. B. A. Eghan Jnr. Date

(Supervisor)

…….

Dr. Robert Ngala

(Head, Department of Molecular Medicine)

ABSTRACT

Several prospective studies have identified hypertension and other conditions such as diabetes mellitus and cardiovascular diseases as strong independent risk factors for renal dysfunction. Little is however, known about the biochemical correlates of renal dysfunction among non-diabetic hypertensives. Moreover, hypertension has been identified as a major contributor to the high morbidity and mortality among African populations and these are sufficiently high to justify viewing the condition as a serious health problem. The overall aim of this study was to evaluate the biochemical correlates of renal dysfunction among non-diabetic, hypertensives. Parathyroid hormone (PTH) has been identified as the main regulator of calcium homeostasis and thus this study set out to evaluate the relationship between PTH and calcium among the study population. Further, this study sought to find out whether a correlation exists between anthropometry as well as blood pressure and the indicators of renal dysfunction among the study population. A total of 252 non-diabetic hypertensives were interviewed for enrolment into the study out of which 200 patients (volunteers) gave their voluntary consent for the study giving a response rate of 79.4%. Another 100 healthy subjects were also recruited as study controls.The results indicates that the prevalence of CKD using MDRD, CKD-EPI, CG-BSA and CG among the hypertensives were 43.0%, 46%, 47.5% and 50% respectively. The hypertensives had significantly higher waist and hip circumferences, they were also heavier and obese (based on their weight and body mass) compared to the control group. Furthermore, the hypertensives presenting with nephropathy were hypocalcaemic and excreted microalbumin as well as protein with none of the control group excreting either microalbumin or protein in the urine.

Stratification between excretion of microalbumin and protein in the urine was 40.5% as against 16% respectively among the study subjects presenting with nephropathy. Significant increases were observed in serum concentrations of urea and creatinine ($p < 0.0001$) whiles serum albumin decreased significantly ($p < 0.0001$) among the hypertensives compared to the control group. Stratification of PTH among study participants and controls showed that serum PTH levels were higher among the study participants compared to the controls and as well increases as the severity of hypertension increases ($p < 0.0001$). The results also show significant increases in serum sodium (Na^+) and chloride (Cl^-) with lower levels of K^+ among the hypertensive group compared to the control group. For every ml min^{-1} 1.73m^{-2} decrease in eGFR ($p < 0.0001$) as estimated by CG, CG-BSA, 4v-MDRD and CKD-EPI, there was a corresponding increase in both systolic and diastolic blood pressures ($p < 0.0001$). Serum PTH, creatinine and urea increased where as serum calcium decreased significantly for every mmHg increase in both systolic and diastolic blood pressures ($p < 0.0001$). The results of the study suggest that parathyroid hormone is linked with derangements in the metabolism of calcium as well as the severity of hypertension and hence may contribute to the development of secondary hyperparathyroidism.

DEDICATION

This work is dedicated to my lovely family, especially Junior and Maame.

ACKNOWLEDGEMENT

I am most grateful to God Almighty for bringing me this far. My heartfelt appreciation also goes to my supervisors, Dr W.K.B.A Owiredu of Molecular Medicine SMS-KNUST and Prof. B.A. Eghan Jnr. of KATH for getting time off their busy schedule to guide me through-out this whole research work. My heartfelt appreciation also goes to Mr Lawrence Quaye and Mr James O Yeboah (JOY) for their immense contribution towards this research work. My sincere gratitude also goes to all my lecturers at the Department of Molecular Medicine, KNUST for their valuable suggestions. Again, I say thank you to the research team especially the nursing colleagues, Dr. F E Brown and the entire laboratory staff of Presbyterian Hospital, Donkorkrom who were with me anytime they were needed. Finally, to all those who contributed in diverse ways to bring this research work to a successful completion.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

ACTH	Adrenocorticotrophic Hormone
BCG	Bromocresol Green
BMI	Body Mass Index
BP	Blood Pressure
CaR	Calcium sensing Receptor
CG	Cockroft Gault
CG-BSA	Cockroft Gault Body Surface Area
CHD	Coronary Heart Disease
CKD	Chronic Kidney Disease
CKD-EPI	Chronic Kidney Disease- Epidemiology Collaboration
CVD	Cardiovascular Disease
DBP	Diastolic Blood Pressure
EASIA	Enzyme Amplified Sensitivity Immunoassay
ECF	Extracellular Fluid
eGFR	Estimated Glomerular Filtration Rate
ESRD	End Stage Renal Disease
GFR	Glomerular Filtration Rate
GLDH	Glutamate Dehydrogenase
GRA	Glucocorticoid Remediable Aldosteronism
HDFP	Hypertension Detection and Follow up Program
HDL-C	High Density Lipoprotein Cholesterol
hPTH	Human Parathyroid Hormone
HRP	Horseradish Peroxidase
IMT	Intima Media Thickness
K/DOQI	Kidney Disease Outcome Quality Initiative
KATH	Komfo Anokye Teaching Hospital
LVH	Left Ventricular hypertrophy
4v-MDRD	4 Variable Modification of Diet in Renal Disease
Mab	Monoclonal Antibodies
MRFIT	Multiple Risk Factor Intervention Trial
NKF	National Kidney Foundation
Pab	Polyclonal antibodies

PAI-1	Plasminogen Activator Inhibitor -1
PTH	Parathyroid Hormone
RAS	Renin Angiotensin Aldosterone System
RHD	Rheumatic Heart Disease
SAP	Systolic Arterial Pressure
SBP	Systolic Blood Pressure
SNS	Sympathetic Nervous System
TPA	Tissue Plasminogen Activator
UAE	Urinary Albumin Excretion
USRDS	United States Renal Data System
VDR	Vitamin D Receptor
WC	Waist Circumference
WHO	World Health Organization
WHR	Waist to hip ratio
WHtR	Waist to Height Ratio

Chapter 1

INTRODUCTION

1.1 INTRODUCTION

Despite its high prevalence, clinical significance and economic cost, hypertensive renal dysfunction is an under recognized and undertreated condition and has been regarded as a public health problem (Bolton and Kliger, 2000, Cappuccio *et al.*, 2004). Studies suggest that non-communicable diseases such as hypertension and its accomplices such as renal dysfunction, cardiovascular diseases, diabetes, etc will soon be the most important cause of morbidity and mortality in the developing world of which Ghana is no exception (Owusu, 2007). According to Horacio and Madias (2007), hypertension affects approximately 25% of the adult population worldwide and its prevalence is predicted to increase by 60% by 2025, where a total of 1.56 billion people may be affected. Essential hypertension, also known as primary hypertension accounts for as many as 95% or more of all cases of hypertension and is a major risk factor for both renal and cardiovascular diseases which is responsible for most deaths (Kaplan, 2006). Estimates based on the Third National Health and Nutrition Examination Survey indicates that 30% of the population in the United States alone aged 17 years or older has some form of renal dysfunction (Coresh *et al.*, 2001). A study on the prevalence of kidney damage in Australian adults indicates that renal function was significantly impaired and highly prevalent among those with hypertension as well as the elderly. It was further reported that proteinuria was five-fold greater in

hypertensive participants compared to their normotensive counterparts (Chaban, 2003).

In Africa, hypertension and its complications including renal dysfunction have been reported and constitute a major factor in the high morbidity and mortality among adults in the Sub-Saharan Africa (Cappuccio *et al.*, 2004). Even though, overall hypertension prevalence is between 10%-15%, prevalence rate as high as 30%-32% have been reported in middle-income urban and some rural areas in Africa (Cooper, 2003). A study carried out in the University of Port Harcourt Teaching Hospital, Nigeria by Onwuchekwa, (2009) revealed hypertension as the commonest cause of congestive cardiac failure. In Ghana, hypertensive renal disease is a common complication especially in the two major cities (Accra and Kumasi) in the country according to studies done by Cappuccio *et al.*, (2004). Again, studies done by Addo *et al.*, (2009) on hypertensive target organ damage among civil servants with hypertension in Accra established a high prevalence (47.5%) of hypertensive target organ damage namely, reduced glomerular filtration rate, hypertensive retinopathy or left ventricular hypertrophy. Available evidence according to Owusu, (2007) also indicates that renal diseases, heart failure and stroke (which are all complications of hypertension) accounted for 23% of acute medical admissions, and 29% of deaths at the Komfo Anokye Teaching Hospital (KATH), Kumasi. Cardiovascular and renal diseases are important contributors to morbidity and mortality among acute medical admissions in Ghana, and among out-patient hypertensives, renal disease is an important complication, particularly among those with consistently high blood pressure. Hypertensive renal

dysfunction is a late complication of hypertension and occurs particularly among black patient populations (Epstein and Oster, 1984). The kidney is one of the major organs that is damaged as a result of long standing elevation in blood pressure. Hypertension may also accelerate atherosclerotic changes that occur in the major arteries of the kidneys. Substantial evidence indicates that hypertension is a major contributor to the development of renal dysfunction in most patients. However such risk ranges from being fairly low in hypertension to a marked increase in susceptibility to hypertensive injury (Griffin, 2006). The impact of hypertension on the progression of renal insufficiency has long been topic of interest. Between 80% and 90% of patients with end-stage renal dysfunction (ESRD) that require dialysis have hypertension. Moreover, there is evidence that early detection and treatment of hypertension can reduce the rate of decline of renal function (Kendal *et al.*, 1995).

JUSTIFICATION

Several prospective studies have identified hypertension and other conditions such as diabetes mellitus and cardiovascular diseases as strong independent risk factors for renal dysfunction. However, little is known about the biochemical correlates of renal dysfunction among non-diabetic hypertensives. Moreover, there is growing evidence that hypertension is a major contributor to the high morbidity and mortality globally, and this is sufficiently high to justify seeing the condition as a serious health threat (Owusu, 2007).

1.2 AIM

The aim of the study therefore, is to evaluate the biochemical correlates of renal dysfunction among non- diabetic hypertensives

1.3 OBJECTIVES

1. To determine the prevalence of renal dysfunction among non-diabetic hypertensives.

2. To examine whether there is a correlation between anthropometry and the indicators of renal dysfunction among these hypertensives.

3. To establish a correlation, if any, between blood pressure and the biochemical indicators of renal dysfunction among the study subjects.

4. To investigate the relationship between calcium and parathyroid hormone among hypertensives.

Chapter 2

LITERATURE REVIEW

2.1 DEFINITIONS AND CLASSIFICATION OF HYPERTENSION

Hypertension or high blood pressure constitutes a repeated elevated blood pressure exceeding 140/90, a condition identified as a major risk factor for cardiovascular mortality and morbidity through its effects on target organs like the kidney, brain and the heart (Cohuet and Struigker, 2006). Hypertension is a characteristic of each individual with marked inter individual variation. The risk of mortality and morbidity rises progressively with increasing systolic and diastolic pressures with each measure having independent prognostic value according to Kumar and Clark (2005).

There are two broad categories of hypertension. Namely, essential (primary) and secondary hypertension with approximately 90-95% of all cases being essential hypertension according to studies done by Klabunde (2005). A small fraction (<5%) of patients with hypertension have diagnosable causes (secondary hypertension). Essential hypertension is currently considered an incurable disorder that requires life-long medical management. Despite many years of active research, there is no unifying hypothesis to account for the pathogenesis of essential hypertension. However, some researchers have propounded a multifactorial aetiology for essential hypertension with both genes and the environment being implicated (Korner, 2007). Pre-hypertension is defined as a systolic BP of 120-139 mmHg and a diastolic BP of 80-89 mmHg. Persons with pre-hypertension progress to overt hypertension at a rate of >10% per year. Lifestyle modifications (salt reduction,

fruits and vegetables, weight reduction, regular exercise) have been shown to reverse pre-hypertension back to normotension.

Grade I or mild hypertension is also defined as a systolic BP of 140-159 mmHg and a diastolic BP of 90-99 mmHg. Grade II or moderate hypertension constitutes systolic BP of 160-179 mmHg and a diastolic BP of 100-109 mmHg. Grade III or severe hypertension constitutes systolic BP of greater or equal to180 mmHg and a diastolic BP of greater or equal to 110 mmHg according to Kumar and Clark, (2005).

High blood pressure genes have not yet been definitely identified, however, research work by Kumar and Clark (2005) has it that high blood pressure tends to run in families, and children of hypertensive parents tend to have higher blood pressure than age-matched children of people with normal blood pressure. The main environmental factors are obesity, high salt intake and mental stress. According to Korner (2007), essential hypertensions occur as two major syndromes: Stress-and-salt related essential hypertension and hypertensive obesity, and each is initiated by chronic stress.

Stress is perceived by the cortex, from which increased dopaminergic neuron activity stimulates the hypothalamic defense area, raising the sympathetic neural activity and blood pressure (Korner, 2007). Normally this subsides quickly when the stress is over, but in those susceptible to essential hypertension, the dopaminergic neuron synapses become sensitized so that the defense response is evoked by ever lower levels of stress (Korner, 2007).

Even though an excess of renin and angiotensin activity could interact with sympathetic nervous system (SNS) to mediate most of its effects, stress may also activate the SNS directly; and SNS overactivity, in turn may interact with high sodium intake, the renin- angiotensin system, and insulin resistance among other possible mechanisms (Norman, 1994).

2.2 EPIDEMIOLOGY OF HYPERTENSION

Worldwide, hypertension is common and is regarded as a public health menace. A recent study on the prevalence of hypertension was found to be 28% in North America and 44% in Western Europe (Wolf-Maier, 2006). Until recently, hypertension was thought to be rare in rural Africa; on the contrary, hypertension and its complications, most notably, renal failure, heart failure and stroke have been reported in black populations all over the world according to studies done by Amoah, (2003).

In Africa, hypertension is now being widely reported and is considered the most common cause of cardiovascular disease on the continent and also a major factor in the high morbidity and mortality of adults in the sub-Saharan Africa (Addo *et al.*, 2009). In some rural and middle income urban areas in Africa, prevalence rate as high as 30 - 32% have been reported (Owusu, 2007). Studies done by Kengne *et al.*, (2007) to provide the current burden on hypertension and its related risk factors in Cameroon established 20.8% prevalence rate and the risk of hypertension significantly increased with clustering of risk factors among the general population. The study further established that non- communicable diseases such as hypertension and its complications will soon outstrip communicable diseases as a

major cause of death in sub-Saharan Africa. This and other researches carried around the globe are in support of the fact that blacks tend to have higher blood pressure than non-blacks, and overall hypertension-related mortality rates are higher among blacks (Gillum, 1996). Moreover, in the Multiple Risk Factor Intervention Trial (MRFIT), which involved more than 23000 black men and 325000 white men followed for 10years, an interesting racial difference was confirmed: the mortality rate of CHD was lower in black men with DBP above 90mmHg than in white men, but the mortality rate of cerebrovascular disease was higher (Neaton *et al.*, 1989). The greater risk of hypertension among blacks suggests that more attention must be given to even low levels of hypertension among blacks (Norman, 1994).

In Ghana, several prospective studies have been carried out to ascertain the extent of the burden especially in Accra and Kumasi. The number of reported new cases of hypertension in outpatient public health facilities in Ghana increased more than ten-fold from 49,087 in 1988 to 505,180 in 2007 (Bonsu, 2010). Over the same period, hypertension relative to the total reported outpatient diseases increased from 1.7% to 4.0% in all ages. In most regions, hypertension ranks as the fifth commonest cause of outpatient morbidity. However, in the Greater Accra Region of Ghana, hypertension moved from fourth to become second to malaria as the leading cause of outpatient morbidity in 2007 (Bonsu, 2010). Stroke and hypertension are among the leading causes of admission and death. Hypertension is an important cause of heart and renal failure in Ghana (Plange-Rhule *et al.*, 1999).

Earlier studies in Ghana revealed a hypertension prevalence of 4.5% among rural dwellers and 8% to 13% in the towns (Cappuccio *et al.*, 2004). More recently, the prevalence of hypertension in urban Accra in a cross-sectional community study establish a crude prevalence rate of 28.3% as well as age standardized rate of 28.4% according to Amoah (2003). Again, a cross-sectional study on 1015 urban civil servants from seven central government ministries in Accra revealed that, out of 219 hypertension participants, 104 representing 47.5% had evidence of target organ damage mainly renal dysfunction as measured by reduced GFR, left ventricular hypertrophy and the presence of hypertensive retinopathy or history of stroke (Addo *et al.*, 2009).

Hypertension is among the most prevalent chronic conditions worldwide; with rates as high as 70% among adults. Studies done by Borzecki, (2009) have also shown that the degree of blood pressure correlates well with the risk for adverse outcomes as well as target organ damage including kidney dysfunction, stroke congestive cardiac failure and development of coronary artery disease. Although absolute cardiovascular risk is based not only on blood pressure level, but also on associated cardiovascular risk factors or target organ damage, individuals with higher blood pressure levels or severe hypertension have a 20-30% ten year risk of cardiovascular disease that increases to a very high risk (30%) in the presence of any risk factor or target organ damage (Borzecki, 2009). Further, these subjects are at high short-term risk for serious cardiovascular events, with the risk increasing with the degree of elevation (Borzecki, 2009).

Women have about the same prevalence of hypertension as men, however, studies on women have shown that they tolerate hypertension better than do men and have lower coronary mortality rates with any level of hypertension (Barrett-Connor, 1997). Thus women are somehow protected against death from coronary heart diseases compared to their male counterparts with comparable coronary risk profiles (Isles *et al.*, 1992). A number of possible explanations have been offered for this protection.

Research work by Ascherio *et al.,* (1996) has it that, less insulin resistance and hyperinsulinaemia because of less upper body fat as well as reduction of blood viscosity and body iron stores by regular menses also confer such protection and that the development of hypertension in women is associated with increasing body weight, alcohol consumption and less high fibre food and magnesium intake.

2.3 HYPERTENSION AS AN INITIATOR OF PROGRESSIVE RENAL DISEASE

Renal dysfunction, both structural and functional is demonstrable in hypertensive individuals even among those with minimally elevated blood pressures according to Fogo *et al.,* (1997). Pathologically, the main changes in milder degrees of hypertension are hyalinization and sclerosis of the walls of the afferent arterioles which is commonly referred to as hypertensive nephrosclerosis (Fogo *et al.*, 1997). Renal involvement is usually assymtomatic with the first objective sign being macroalbuminuria, serving as a marker for impaired intrarenal vasodilatory responsiveness, and also a likely factor in the initiation and progression of tubulointestitial damage (Norman, 1994).

Most easily assessed by measurement of the albumin:creatinine ratio, microalbuminuria is a predictor not only for progressive renal damage but also of overall cardiovascular morbidity (James *et al.,* 1995). According to Klag *et al.,* (1995), even though a small minority of hypertensives develops progressive renal insufficiency, black hypertensives develop more end stage renal dysfunction, possibly due to limited access to health care. The kidney is one of the major organs that is damage as a result of long standing hypertension and has been shown to play an important role in blood pressure regulation. The kidney is a main target of organ damage in hypertension, and long-term exposure to elevations in blood pressure (BP), even within the normotensive range, can induce early renal damage. Current expert guidelines for the management of hypertension recommend determination of the serum creatinine concentration in all patients with hypertension as a marker of target organ damage according to WHO international society guidelines for the management of hypertension, (1999).

In the Joint National Committee VI guidelines, a frankly elevated creatinine concentration or the presence of proteinuria is considered a sign of organ damage, and a creatinine level of 106 to 178 μmol/L (1.2-2.0 mg/dL) is a major tool for risk stratification according to the World Health Organization–International Society of Hypertension guidelines. Nevertheless, few data exist about the prognostic value of normal or minimally elevated creatinine levels in hypertension. Despite the fact that renal hemodynamic values become abnormal even in the early stages of hypertension, the glomerular filtration rate is usually not significantly reduced until late in the course of the disease (Cocoran *et al*., 1948, Perera, 1995).

Elevated serum creatinine level is therefore a late sign of renal damage in essential hypertension. Although not an ideal marker for renal function, an increased serum creatinine level is strongly predictive of the subsequent development of end-stage renal disease according to Perrone (1992) and Iseki (1997).

Moreover, overt elevated serum creatinine values predict a poor prognosis in patients with hypertension (Sokolow and Perloff, 1961., Shulman *et al.*, 1989), whereas mild elevations in serum creatinine levels were associated with an increased all-cause mortality rate in population-based samples of elderly patients and in patients with heart failure (Psaty *et al.*, 1996). A study done by Willem (1990) indicates that, transplantation of a kidney from a normotensive donor into a patient with nephrosclerosis can lead to remission of essential hypertension. This was shown using the Milan strain of hypertensive rat, in which transplantation of a kidney from a normotensive rat induced a fall in blood pressure. Although renal damage is difficult to detect in the early stages of the development of hypertension, arteriosclerosis has been found in the majority of hypertensives who have had the disease for sometime (Fishberg, 1927).

In patients with severe or accelerated hypertension, and in patients with essential hypertension, proteinuria as well as evidence of kidney function impairment is common. It is also known that early intervention and antihypertensive treatment stops this and resolves the necrotizing lesion (Breckenridge, 1971). However, some patients with essential hypertension who are treated develop intimal fibrosis of the interlobular arteries which may lead to progressive renal failure; even though at the beginning of treatment the patient did not have an elevated creatinine level

(Dustan *et al.,* 1958). The majority of patients who do not have gross evidence of necrotizing arteriolitis and who develop end-stage kidney dysfunction do so because of progressive arteriolosclerosis (Jacques *et al.,*1983). It is well known that the kidney may be both a cause and a victim of high blood pressure, and as to whether systolic or diastolic blood pressure poses a greater risk for renal function deterioration has become a challenge to most researchers.

The modification of diet in renal disease study showed that subjects with mean diastolic blood pressure of 100 mmHg or more exhibited a significantly greater decline in renal function than subjects with diastolic pressure below this level (Klahr, 1994). In another retrospective study of 86 patients on dialysis, 50% of whom were blacks; Brazy *et al.,* (1989) found a positive correlation between mean diastolic blood pressure and rate of decline in renal function. Research work done by Plange-Rhule *et al.,* (1999) on the burden of renal diseases among out-patient hypertensives in Kumasi revealed that, among 448 hypertensive patients, 30.2% had plasma creatinine above the reference range with 25.5% having proteinuria. In the Hypertension Detection and Follow–up Program (HDLP), the group with the highest blood pressure at entry had the highest serum creatinine (Norman, 1994).

Similarly, the decline of renal function was greatest in those with blood pressure of 140/90 mmHg or greater compared to those who were either normotensive or hypertensive with adequately controlled blood pressure according to Norman (1994). However, Cerasola *et al.,* (2010) recently identified systolic rather than diastolic blood pressure as an independent risk factor in renal function deterioration. In multiple regression analysis, Cerasola *et al.,* (2010) were able to

show that the probability of having stages 1 and 2 CKD was significantly higher in subjects with greater values of systolic blood pressure and with higher waist circumference. Systolic blood pressure was also positively related to stage 3 CKD, whereas stages 4 and 5 correlates inversely with waist circumference.

2.4 MICROALBUMINURIA IN HYPERTENSION (RENAL AND CARDIOVASCULAR IMPLICATIONS).

Microalbuminuria refers to low but abnormal levels of albumin equal to 30mg/day in the urine or urinary albumin excretion rate (UAE) of >0.29nmol/min (20µg/min) according to Onovughakpo-Sakpa *et al.,* (2009). It has recently been recognized as a predictive as well as an early marker for impending nephropathy and a more aggressive cardiovascular complication in hypertensive subjects, and has also been regarded as an independent risk factor for both renal and cardiovascular damage (Andronico, 1998). Patients with microalbuminuria are referred to as having incipient nephropathy, and without specific intervention, 20-40% of such patients especially; those with type 2 diabetes in addition progress to overt nephropathy (Onovughakpo-Sakpa *et al*., 2009).

Studies done by Luft (1997), indicates that increased blood pressure, age, smoking habit and diabetes appears to be the most important factors contributing to microalbuminuria, particularly among hypertensives. It was further reported that microalbuminuria is potentially reversible if detected early, and that all antihypertensive agents appear to reduce microalbuminuria particularly angiotensin-converting enzyme inhibitors therapy which is effective in reducing renal disease progression especially in diabetics. According to Pontremoli (1996),

microalbuminuria has been associated with a number of unfavorable metabolic and non-metabolic risk factors, such as older age, longer duration of hypertension, cigarette smoking, increased BP load and variability, higher uric acid levels, worse lipid profile, insulin resistance, endothelial dysfunction, increased activity of the RAS, and BP salt sensitivity.

Tuttle *et al.,* (1999), showed a correlation between increased UAE and the severity of coronary heart disease at angiography. In another study, Berton *et al.,* (1998) showed that the presence of microalbuminuria strongly predicts mortality in patients with acute myocardial infarction, even after adjusting for several other confounders, such as age, the presence of hypertension, heart failure, and serum lipids. Microalbuminuria has also been related to peripheral atherosclerosis and increased carotid intima-media thickness (IMT) in some but not all studies (Pontremoli *et al.*, 1998). In light of the well-known association between carotid atherosclerosis and cerebrovascular damage (both asymptomatic vascular lesions and acute events), it is not unexpected that microalbuminuria has been shown to be a predictor of ischemic stroke, even after correction for the presence of several confounding factors (Beamer, 1999).

Apart from the renal and cardiovascular implications associated with the presence of microalbuminuria, it has been well elucidated that microalbuminuria particularly among essential hypertensives is associated with other signs of subclinical organ damage such as left ventricular hypertrophy, hypertensive retinopathy and increased aortic stiffness according to Giorami *et al.,* (2008).

Several population studies have evaluated the prevalence of non-diabetic microalbuminuria with results ranging from 1 to 56% (Pedrinelli *et al.*, 2002). Several factors can affect the prevalence of microalbuminuria in hypertension, including severity of the disease, selection procedures, concomitant risk factors, degree of obesity, age, and sex distribution. This accounts for the large differences in the prevalence of microalbuminuria that can be found in different studies, with prevalence rates going from a low of 4.7% to a high of 40% (Rosa and Palatini, 2000). A study done by Onovughakpo-Sakpa *et al.,* (2009) on the incidence of diabetic nephropathy among Southern Nigerians showed that the incidence of microalbuminuria amongst diabetics is 72.63% with increased albumin-creatinine ratio. The study further showed that microalbuminuria correlates positively with duration of diabetes.

Research work by Sierra *et al*., (2000) to evaluate the clinical and biochemical profile as well as its association with microalbuminuria in a group of essential hypertensives established 22.3% prevalence of microalbuminuria among the study population. The study further concluded that, among essential hypertensive patients, the presence of microalbuminuria is associated with increased blood pressure. Likewise, microalbuminuria is associated with the degree of renal impairment, and with increased triglycerides as well as decreased HDL cholesterol. Further, studies done by Seema *et al.,* (2008) in a subgroup of 376 never-treated essential hypertensives aged 40 years and above indicates overall presence of 16% microalbuminuria and futher suggested microalbuminuria as a good predictor of left ventricular hypertrophy (LVH).

2.5 MICROALBUMINURIA VERSUS PROTEINURIA AMONG HYPERTENSIVES

Hypertension is a well known risk factor associated with high cardiovascular risk as well as kidney disease progression (Bakris, 2005). The spectrum of albuminuria, from microalbuminuria (>30mg/day but <300mg/day) to proteinuria (>300mg/day) is associated with a linear increase in the risk of cardiovascular events. Studies have shown that microalbuminuria correlates with the magnitude of C-reactive protein elevations and has also been associated with a failure of nocturnal drops in arterial pressure, insulin resistance as well as abnormal vascular responsiveness to a variety of stimuli. The presence of microalbuminria therefore indicates abnormal responses by vascular tissue, perhaps due to the underlying inflammatory responses. These data therefore supports the concept that microalbuminuria is associated with increased cardiovascular risk and proteinuria represents even higher cardiovascular risk with high risk for progression to end-stage kidney disease (Ford, 2003, Stuvelling *et al.*, 2004).

Proteinuria signifies a membrane barrier defect not only in the podocytes and vascular endothelium of the kidney but also in the vascular tissues throughout the body. The major contribution to this defect is through sustained long term elevations in blood pressure and resultant barrier disruption (Bakris, 2005). Acute profound reductions in blood pressure are associated with small reductions in proteinuria over a short period of time; however, these reductions become more pronounced over longer periods of follow-ups and are also associated with reductions in cytokines productions which emphasize the need for blood pressure

control and regular check-ups among hypertensive's (Braam *et al.*, 2005). Studies done by Agarwal and Anderson (2005) established among non-diabetic male cohort with eGFR that the log of urine protein : creatinine ratio was the strongest predictor systolic blood pressure elevation, with the strength of relationship between proteinuria and systolic blood pressure being the highest for ambulatory blood pressure and lower for routine clinic blood pressure. Thus in the study, proteinuria was the strongest and most important correlate of systolic blood pressure. Onovughakpo-Sakpa *et al.,* (2009) found that patients with reduced renal functional reserve capacity may be more prone to microalbuminuria when exposed to conditions such as hypertension. Thus hypertension is a potent precipitant of impending renal dysfunction which is manifested by microalbuminuria. The study further indicates that microalbuminuria constitutes a reversible state and can be resolved by specific medications such as angiotensin converting enzyme inhibitors and angiotensin receptor blockers.

Martins *et al.,* (2002), showed in an epidemiological data that blood pressure is directly linked to CKD and proteinuria as well as kidney disease related mortality. Proteinuria and evidence of renal function impairment are grave signs in the hypertensive person. Moreover, the presence of proteinuria and renal function impairment worsens the prognosis of the patient with essential hypertension. Besides, arteriolonephrosclerosis and necrotizing arteriolitis of the hypertensive, other lesions seen in the renal circulation of the hypertensive person are due to intimal fibrosis of the interlobular arteries and these may lead to progressive renal damage (Jacques *et al.*, 1983). The risk of ESRD, at least among African-Americans

and whites is inversely related to socioeconomic status and directly related to blood pressure levels (Flack *et al.,* 2002). Approximately 85% of persons with CKD have hypertension and patients with proteinuria superimposed on CKD have higher blood pressure than those with non- proteinuric CKD (Flack *et al.*, 2002).

2.6 CHRONIC KIDNEY DISEASE (CKD) AND RENAL DETERIORATION AMONG HYPERTENSIVE PATIENTS.

Chronic kidney disease (CKD) is a serious condition associated with premature mortality, decreased quality of life and increased health-care expenditure. Increasing evidence accrued in the past indicates that the adverse outcomes of CKD such as kidney failure, CVD and premature death can be prevented if earlier stages of the disease could be detected through laboratory testing (National Kidney Foundation, 2002). Given the pathogenic progression of kidney disease, patients with CKD are at high risk for progression to the end stage renal disease (ESRD) – a condition requiring dialysis or kidney transplantation to maintain patients' long-term survival (Schoolwerth *et al.*, 2006).

Moreover, current evidence suggests that hypertension and diabetes are the two major causes of chronic kidney disease worldwide (Gilber *et al.*, 2005), even though several established or suspected risk factors such as dyslipidemia, albuminuria, old age, chronic anaemia, oxidative stress, certain drugs, race and low socioeconomic status among others have been associated with the occurrence and progression of CKD according to Mitch (2007).

According to the morbidity and mortality report from the United States centre for disease control and prevention, CKD was more prevalent among persons with hypertension than among those without hypertension (24.6% vs. 12.5%), (Coresh *et al.,* 2001)

Again, 70% of patients with elevated serum creatinine (defined as greater or equal to 1.6mg/dl for men and 1.4mg/dl for women) have hypertension according to studies done by Coresh *et al.,* 2001. Further, 26.8% of patients with end-stage renal disease (ESRD) were deemed to have kidney failure as a result of hypertension according to data available at the USRDS (2008). Available facts from the National Kidney Foundation (2002) indicates that several millions of adults in the United States alone have CKD, and several million others are also at increased risk and that, hypertension and diabetes still remain the major risk factors accounting for up to two-thirds of all cases of CKD. These and other supporting facts presupposes that high BP is a common cause of CKD and hence patients with high BP are at increased risk of loss of kidney function and development of CVD.

Individuals with high BP should therefore be carefully evaluated for the presence of CKD especially those with decreased GFR. Although there are few existing data on the prevalence of CKD and its accompanying complications in Africa, the condition is thought to be on the increase. A multicenter screening study done by Osafo *et al.*, (2011) to identify the prevalence and staging of CKD among 712 known hypertensives in four polyclinics in the capital city of Ghana revealed that, CKD is common among hypertensives in Ghana, with a prevalence rate of 46.9%.

High blood pressure is a major promoter of the decline in glomerular filtration rate (GFR) in both diabetic and non-diabetic kidney disease, however, only a small percentage of patients with hypertension will develop CKD (Freedman and Sedor, 2008). According to the National Kidney Foundation Kidney Disease Outcome Quality Initiative (K/DOQI), CKD constitutes kidney damage for greater or equal to 3 months, as defined by structural or functional abnormalities of the kidney with or without decreased GFR, and manifest by pathological abnormalities or markers of kidney damage, including abnormalities in the composition of the blood or urine, or abnormalities in imaging tests. Chronic kidney disease is therefore defined as kidney damage identified by proteinuria or by GFR of less than 60ml/min per 1.73m^2 body surface area with or without evidence of kidney damage for 3 months or longer (Levy *et al.*, 2003). The disease process begins with renal hypertrophy and hyperfiltration resulting from elevated renal plasma flow. Hyperfiltration is typically followed by the loss of negatively charged glomerular filtration barrier allowing for negatively charged proteins such as albumin to pass through the glomerulus and into the urinary space. The presence of these proteins in the urinary space elevates urinary albumin and produces microalbumin which consequently can result in macroalbuminuria or nephritic range proteinuria (>3.5g/24hrs) (Trevisan and Viberti, 2000).

Once macroalbuminuria (frank proteinuria) sets in, GFR begins to decline and progressive mesangial and interstitial capillary occlusion then occur, restricting the glomerular filtration surface and leading to a further decrease in GFR. Some proteins are reabsorbed by the renal tubules and accumulate in tubular epithelial

cells. This accumulation induces the release of vasoactive and inflammatory cytokines, which damage the renal tubules and lead to tubular atrophy as well as interstitial fibrosis (Gruden *et al.,* 2004).

2.7 THE ROLE OF SODIUM, POTASSIUM AND CALCIUM IN HYPERTENSION.

Hypertension results from the interplay of both the internal and external environment. Numerous studies have shown adverse effects of a surfeit of sodium on arterial pressure, with potassium being viewed as a minor factor (Horacio and Medias, 2007). Abundant evidence however, indicates that potassium deficit has a critical role in hypertension and its cardiovascular sequelae. Research work by Horacio and Medias (2007) indicates that increasing the potassium intake of hypertensive rats that were fed with high sodium diet lowered blood pressure, reduced the incidence of stroke and stroke related death, cardiac hypertrophy and as well as renal injury. In 2002, du Cailar *et al,* assessed the influence of sodium intake on the relationship between systolic arterial pressure (SAP) and target organs (i.e, left ventricular mass index and albumin excretion rate) in a large cohort of 839 normotensives and never- treated hypertensive subjects aged 15-70 years. It was observed that increasing dietary sodium was associated with an increasingly steeper slope of the relationship of SAP versus AER or left ventricular mass index. Again, the prevalence of left ventricular hypertrophy or microalbuminuria was higher in hypertensive patients aged 40 years and above. These and other studies done elsewhere supports the assertion that potassium supplementation can reduce the need for antihypertensive medication.

According to Horacio and Medias (2007), one study showed that, with an increased dietary potassium intake in hypertensive subjects, 81% of the subjects needed less than half of the baseline medication and 38% require no antihypertensive medication for blood pressure control at one year follow-up. Research work by Kappanen (1991), suggest that without sodium chloride and other sodium compounds being added to diet, arterial hypertension would be non-existent. Results from Kappernen's study further established that, in communities with a high consumption of added salt, a high intake of potassium and possibly, magnesium seem to protect against the development arterial hypertension as well as a rise in blood pressure with age. Sodium levels are higher in hypertensive subjects than their normotensive counterparts and especially, in association with a rise in blood pressure (Lionel and Yackoob, 2005). Reabsorption of filtered sodium by the renal tubules is increased in subjects with essential hypertension due to the stimulation of several sodium transporters located at the luminal membrane as well as the sodium pump which is localized at the basolateral membrane which provides the energy for such transport (Horacio and Medias, 2007).

According to the INTERSALT research study across populations, mean systolic blood pressure was 5mmHg higher and diastolic blood pressure was 3mmHg higher when sodium intake was increased by 50mmol/day. In analysis within single population, a positive correlation between sodium intake and blood pressure was also detected according to Horacio and Medias (2007). According to the body sodium hypothesis (which states that body sodium may be normal in subjects with essential hypertension, but the response of arterial pressure to

changes in body sodium is abnormal), mean total exchangeable sodium is normal in subjects with essential hypertension and yet amongst such patients there is a positive correlation of arterial pressure and exchangeable sodium, a relation which is not found in normal subjects (Jacques *et al.,* 1983). Calcium is also effective in reducing blood pressure in various states of hypertension including pregnancy-induced hypertension and pre-eclampsia (Pfeifer *et al.*, 2001). According to Mariano *et al.,* (2006), low calcium levels as well as calcitriol are important factors involved in the development of secondary hyperparathyroidism in subjects with chronic kidney disease which may be due to long standing hypertension. This then presupposes that, low calcium levels, vitamin D deficiency and accumulation of phosphate due to decrease renal function are the main pathogenic factors involved in the pathogenesis of secondary hyperparathyroidism in patients with chronic renal dysfunction.

Studies done by St. John *et al.*, (1994) to investigate the relationship between calcitrophic hormones and blood pressure among the elderly who were untreated for hypertension in a univariate analysis showed that serum parathyroid hormone as well as 1,25-dihydroxycholecalciferol correlated significantly with mean blood pressure. A multivariate analysis in the same study also demonstrated that serum parathyroid hormone and 1,25- dihydrocholecalciferol were both independent determinants of mean blood pressure. Research work by Aloia *et al.,* (2006) indicates that increased parathyroid hormone significantly correlates with increased BMI, serum creatinine and age. The study also depicts that serum

parathyroid hormone was significantly higher in black study subjects than white study subjects.

Studies done by several researchers including that of Young *et al.,* (1990) and Resnick *et al.,* (1986) indicates that, there is association between hypertension, calcium, vitamin D and parathyroid hormone. Moreover, alterations in calcium metabolism have been demonstrated in human essential hypertension. Indeed, studies done by Young *et al.,* (1990), indicates that, a direct correlation actually exist between essential hypertension and serum parathyroid hormone in men and that, hypertensive men had parathyroid hormone levels that were 36% higher than normotensive men. Moreover, patients with essential hypertension have been reported to have higher serum concentration of parathyroid hormone than their normotensive counterparts, although this finding is not universal among studies according to Young *et al.*, (1990).

Serum calcium levels is tightly regulated by parathyroid hormone and thus, a small decrease in serum calcium elicits a prompt increase in the secretion of parathyroid hormone, which in turn mobilizes calcium from the skeleton and increases the renal tubular reabsorption of calcium (Rolf *et al.*, 2000). Furthermore, parathyroid hormone stimulates the hydroxylation of 25-hydroxycholecalciferol to the more biologically potent 1,25-dihydroxycholecalciferol in the kidneys which again leads to an increase absorption of calcium from the intestine(Bouillon *et al.*, 1997). Hypocalcemia may therefore develop primarily from decreased intestinal

calcium absorption because of low plasma calcitriol levels and possibly from calcium binding to elevated serum levels of phosphate.

2.8 DEVELOPMENT OF SECONDARY HYPERPARATHYROIDISM IN RENAL DYSFUNCTION

Disturbances in calcium, phosphate and vitamin D metabolism in chronic renal insufficiency play a major role in the development of secondary hyperparathyroidism. This not only causes bone diseases (renal osteodystrophy), but also contributes significantly to the high cardiovascular mortality among such patients (Amann *et al.*, 1999). Calcifications of coronary plaques, cardiac valves and myocardial tissue, as well as diffuse myocardial fibrosis are common pathologic finding (Goodman *et al.*, 2000). Similarly, increased mortality has recently been observed in patients with high parathyroid hormone levels. A very important feature of the parathyroid gland is its high sensitivity to small changes in serum calcium concentration (Slatopolsky, 1998).

A reduction in extracellular calcium concentration leads immediately to an increase in parathyroid hormone secretion and subsequently stimulates an increase in parathyroid hormone synthesis and this act consequently enhances parathyroid cell proliferation (Silver, 2000). The mechanism responsible for secondary hyperparathyroidism has advanced significantly and it appears that in early stages of renal dysfunction, a deficit of calcitriol synthesis is an important factor, however, additional factors such as a deficit of the vitamin D receptor or the newly cloned calcium sensor (BoPCaR1), may be present in the parathyroid cells.

Consequently, as renal dysfunction progresses, the lack of calcitriol become more pronounced, and thereby, inducing secondary hyperparathyroidism.

In advance chronic renal failure, hyperphosphatemia is an additional important factor in worsening secondary hyperparathyroidism as well as resistance of the parathyroids to calcitriol due to a reduced density of calcitriol receptors (Llach, 1995). It has also been postulated that hyperparathyroidism in chronic renal dysfunction results from hypocalcemia, occurring in part, from phosphate retention and or deficit in 1, 25-dihydroxycholecalciferol synthesis. Low serum calcitriol levels, hypocalcemia, and hyperphosphatemia have all been demonstrated to independently trigger PTH synthesis and secretion. As these stimuli persist in renal insufficiency, particularly in the more advanced stages, PTH secretion becomes maladaptive and the parathyroid glands, which initially hypertrophy, become hyperplastic. The persistently elevated PTH levels exacerbate hyperphosphatemia from bone resorption of phosphate.

Studies done by Pitts *et al.,* (1988) to investigate hyperparathyroidism and 1,25-dihydroxycholecalciferol deficiency in a group of patients with mild, moderate and severe renal dysfunction indicates that subjects with mild renal dysfunction had normal mean serum ionized calcium and phosphorus but with a decrease mean 1,25-dihydroxycholecalciferol compared to the control subjects. However, in subjects with moderate renal impairment, even though plasma ionized calcium was normal, plasma PTH levels were elevated with a more pronounced decrease in 1,25-dihydroxycholecalciferol. Among the final group of subjects with severe

renal impairment, plasma ionize calcium was decreased with a marked elevation in plasma PTH levels.

These findings therefore demonstrate the presence of hyperparathyroidism, normocalcemia and hypocalcemia as well as 1,25-dihydroxycholecalciferol deficiency among subjects with various degrees of renal impairment.

2.8.1 ***Role of vitamin D***

In chronic renal insufficiency, a decreased number of vitamin D receptors (VDR) and resistance to the action of vitamin D are of great importance in the pathogenesis of secondary hyperparathyroidism (Drueke, 1995). This phenomenon is far more marked in the nodular than in the diffuse forms of parathyroid hyperplasia (Fukuda, 1993). In chronic renal insufficiency, VDR are down regulated. The mechanism seems to be post-transcriptional (Slatopolsky *et al.*, 1996).

As a result, the low serum $1,25(OH)_2D3$ level and parathyroid VDR number lead to a stimulation of PTH gene expression. In addition, the heterologous regulation of the calcium sensing receptor (CaR) expression probably involves also $1,25(OH)_2D3$ and phosphorus. Thus, vitamin D deficiency and phosphorus retention may reduce CaR-mRNA. There is also evidence that $1,25(OH)_2D3$ can regulate parathyroid cell proliferation directly (Drueke, 2000).

2.9 BONE DISEASE IN CKD

Bone disease associated with CKD is composed of a number of abnormalities of bone mineralization. The major disorders can be classified into those associated with high bone turnover and high PTH levels (including osteitis fibrosa, the hallmark of secondary hyperparathyroidism), low bone turn over and low or normal PTH levels (osteomalacia and adynamic bone disease). Osteomalacia may be related to vitamin D deficiency, excess aluminium, or metabolic acidosis; whereas a dynamic bone disease may be related to oversupression of PTH with calcitriol.

The pathophysiology of bone disease due to secondary hyperparathyroidism is related to abnormal mineral metabolism as a result of the following: 1) Decrease kidney function which leads to a reduced phosphorus excretion and hence, its consequent excretion; 2) Elevated serum phosphorus which can directly suppress calcitriol (Dihyroxyvitamin D3) production; 3) Reduced kidney mass which ultimately leads to a decrease calcitriol production; 4) Decreased calcitriol production with a consequent reduced calcium absorption from the gastrointestinal tract which contribute to hypocalcemia (Francisco,1995).

Hypocalcemia, reduced calcitriol synthesis and elevated serum phosphorus levels stimulate the production of PTH and the proliferation of parathyroid cells resulting in secondary hyperparathyroidism. The hallmark lesion of secondary hyperparathyroidism is cysteitis fibrosa cystic. High bone turnover leads to irregularly woven abnormal osteoid fibrosis as well as cyst formation. This result

in decreased cortical bone and bone strength and an increased risk of fracture (Francisco, 1995).

2.10 ASSOCIATIONS WITH HYPERTENSION

In addition to other possible mechanisms, surveys of large populations have revealed a number of associations with hypertension that are likely not directly causal but are reflective of shared mechanisms or of consequences of hypertension according to research work done by Cannon *et al.,* (1996). Hyperuricaemia is found in as many as half of untreated hypertensives and that gout is more common (Cannon *et al.*, 1996). Whereas this may reflect nephrosclerosis as well as reduced renal function, other possible reasons according to Cappuccio *et al.,* (1993) include an increased proximal tubular reabsorption of uric acid in concert with sodium, an effect of upper-body obesity, insulin resistance and hypertriglyceridemia and contribution of excessive alcohol intake (Bonora *et al.*, 1996).

2.11 HAEMATOLOGIC FINDINGS AMONG HYPERTENSIVES

2.11.1 ***Red cells***

Higher haematocrits are found in hypertensives; and the prevalence of hypertension doubles with an increase of 10% in the haematocrit according to Cirillo *et al.,* 1992). Again among young subjects in the Tecumseh study, higher haematocrits were associated with higher blood pressure, weight, glucose, cholesterol and insulin levels (Smith *et al.*, 1994). Studies done by Wannamethee *et al.,* (1994) among middle- aged subjects in England indicates that the risk of stroke was three times higher in hypertensives with haematocrit above 51% than those with lower haematocrit.

2.11.2 *White Cells*

Elevated white blood cell counts are predictive of the development of hypertension (Friedman *et al.*, 1990) and are as well likely related to insulin resistance and hyperinsulinaemia (Facchini *et al.*, 1992). In the Framingham population, higher white blood cell counts were associated with increased risk of cardiovascular disease according to studies done by Kannel *et al.,* (1992).

2.11.3 *Fibrinogen and Hypofibrinolysis*

Elevated plasma fibrinogen levels is a major risk factor coronary heart disease (Yarnell *et al.*, 1991) and have been noted along with increased plasminogen-activator inhibitor 1 (PAI-1) levels in hypertensives with insulin resistance (Landin *et al*, 1990). Decreased fibrinolytic activity along with higher PAI-1 and lower tissue plasminogen activator (TPA) activity have been found in patients with hypertension and hypercholesterolemia (Jansson *et al.*, 1991). Research work done by Wall *et al.,* (1995) indicates that, young men with borderline hypertension and normal metabolic status had higher levels of TPA antigen, also known to be a risk factor for coronary thrombosis.

2.12 HYPERTENSION AND THE HEART

An earlier study done by Kannel *et al.,* (1972) indicates that patients with essential hypertension develop congestive heart failure and myocardial infarction at an increased rate, although congestive heart failure has been largely eliminated in the well- treated essential. However, myocardial infarctions continue to be a major problem for the hypertensive and most likely the single largest cause of death in even the treated hypertensive patient.

It has been shown that systolic together with diastolic pressure, mean blood pressure, pulse pressure and systolic pressure have a direct correlation with the development of congestive heart failure (Jacques *et al.*, 1983). Several aetiological factors have been established for heart failure in West Africa. Notable among these aetiological factors include hypertension, cardiomyopathy, peripartum cardiac disease and rheumatic heart disease (RHD). Research work carried out by Ladipo *et al.,* (1997) in Nigeria to ascertain the patterns of heart disease in adults established hypertension as one of the leading and major causes of heart failure. Anthony (1980) also studied 315 cases of heart failure admitted to the Katsina General Hospital in Northern Nigeria and found out that hypertension accounted for 12% of heart failure among the studied population.

Recent studies provide important and worrisome findings as far as the clinical outcomes of hypertension are concerned. Hypertension has been reported to account for up to 30% of hospital admissions for heart failure alone in the West African sub region (Toure *et al.,* 1992). Moreover, the prognosis of hypertensive heart failure among black African populations has also been found to be poor according to studies done by Isezuo *et al.*, (2000). Consecutive study on 572 patients with heart failure to the National Cardiothoracic Centre, Accra, Ghana over a four-year period evaluated for the aetiology using a 2-dimensional Doppler echocardiography with a colour flow established hypertension as the major cause of heart failure according to Amoah and Kallen, 2000.

MacMahon (1990) performed a meta-analysis of all available major prospective observational studies relating diastolic blood pressure (DBP) level to the incidence of stroke and coronary heart disease (CHD). Out of the nine studies analyzed, almost 420,000 people were followed up for 6-25 years, and a total of 599 fatal strokes as well as 4260 deaths from CHD were recorded.

2.13 ENDOCRINE ASSOCIATIONS

2.13.1 ***Role of corticosteroids in hypertension***

Although the underlying pathophysiology remains incompletely understood, it is assumed that essential hypertension has a multifactorial cause and that no single cause exists. However, a number of studies draw attention to the adrenal cortex and its contribution to BP elevation. Functional abnormalities of the adrenal cortex were suggested as a cause for essential hypertension many years ago (Genest *et al.*, 1956); indeed, early studies reported that adrenal cortex hyperplasia was a feature of many hypertensive individuals at post mortem examination (Komiya *et al.*, 1991).

In addition, a number of abnormalities of urinary excretion, plasma levels, and clearance of several adrenal steroids in patients with hypertension have been identified over the years (Honda *et al.*, 1975). No single defect in adrenal corticosteroid biosynthesis has been identified, however, it is important to consider briefly two rare monogenic syndromes involving 11-beta-hydroxylase and aldosterone synthase that cause hypertension and help identify candidate mechanisms. Research work by Burt *et al.*, (1995) indicates that Glucocorticoid remediable aldosteronism (GRA) is a rare autosomal dominant condition

characterized by hypertension and aldosterone excess that is regulated by ACTH rather than Ang II. The molecular basis of this condition was first described in 1992 according to research work by Lifton *et al.*, (1992). A chimeric gene that contains the 5' promoter sequence of CYP11B1 and functional elements of CYP11B2 is created, resulting in aldosterone production under control of ACTH according to Lim *et al.*, (1999). Deficiency of 11-beta-Hydroxylase is a rare cause of congenital adrenal hyperplasia, accounting for 5 to 8% of cases (White, 2001). In this autosomal recessive disorder, mutations in CYP11B1 result in impaired activity of 11beta-hydroxylase, leading to accumulation of the steroid precursors 11-deoxycortisol and deoxycorticosterone, leading to mineralocorticoid hypertension in approximately two thirds of cases (Honda *et al.*, 1975).

In light of these rare syndromes, it is of interest that impaired activity of the enzyme 11-beta-hydroxylase has also been reported to be a feature of patients with essential hypertension (de Simone *et al.*, 1985). More recently, a similar phenomenon was observed in patients with hypertension from Italy, in whom the ratio of 11-deoxycortisol to cortisol (a marker of 11beta-hydroxylase activity) was elevated (Connell *et al,* 1999). Although the precise cause of this is unclear, it was suggested that it may be a consequence of variation at the CYP11B1/CYP11B2 loci, which encode 11-beta-hydroxylase and aldosterone synthase, respectively.

2.13.2 *Mineralocorticoid excess and its association with hypertension*

Mineralocorticoides stimulate the distal renal tubules to reabsorb sodium from tubular fluid and excrete more potassium and hydrogen ions. They increase open sodium and potassium channels in the luminal of tubular cells and increase synthesis of basolateral membrane Na+/K+ ATPase, which generate the gradients that drive ion movement (White, 2001). Mineralocorticoides expand extracellular fluid volume by increasing the amount of in the body, and increase blood pressure due to greater intravascular volume and increased arteriolar resistance. They thus lower plasma potassium levels and increase plasma pH. The mineralocorticoid receptor is activated by both cortisol and aldosterone (White, 2001).

Aldosterone is the primary mineralocorticoid because an enzyme (11-beta hydroxyl steroid dehydrogenase) coexists with this receptor in the renal tubule and coverts cortisol to inactive cortisone. Hereditary defects or drug inhibition of this enzyme produces a syndrome of apparent mineralocorticoid excess, due to receptor activation by normal levels of cortisol (Connell *et al.,* 1999). Licorice contained in some candies and tobacco products has a metabolite (glycyrrhetinic acid) that produces mineralocorticoid excess by inhibiting 11-beta hydroxysteroid dehydrogenase (Connell *et al.,* 1999).

Chapter 3

MATERIALS AND METHODS

3.1 STUDY SITE

The Kwahu North (Afram Plains) is one of the 21 Districts in the Eastern region with a population of about 135,928 people made up of 72624 males and 63254 females. The District covers a land size of about 5040sq.km out of the 19,323sq.km land of the Eastern region, making it the largest district in the region. About 80% of the population are farmers. Donkorkrom is the District capital. Presbyterian Hospital, Donkorkrom is the only District Hospital in the Kwahu North District. It is the only hospital serving the larger populace in the Kwahu North District in the Eastern region of Ghana. It also serves as a referral point for the numerous clinics, Health centre's and the CHPS in and around the Kwahu North District (KNDA, 2010).

3.2 STUDY DESIGN

The study was conducted between August 2010 and September 2011 among 200 non-diabetic hypertensives. To qualify for recruitment, subjects must be non-diabetic hypertensives aged not less than 18years and not more than 70 years with fasting blood glucose not greater than 7.0mmol/L. A total of 252 non - diabetic hypertesives were interviewed out of which 200 patients representing 79.34% gave their conscent for the study. Another one hundred (100) healthy normotensives were also recruited as controls. Written informed conscent was obtained from each participant. The study was approved by the Committee on Human Research Publication and Ethics.

3.3 MEASUREMENT OF ANTHROPOMETRIC VARIABLES

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

3.3.1 Blood Pressure

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

3.4 URINALYSIS

Early morning urine was collected into plastic universal containers for microalbuminuria and proteinuria using the dipstic qualitative method.

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

3.5 SAMPLE COLLECTION AND PREPARATION

Venous blood samples were collected after an overnight fast (12-16 hrs). About 6mls of the blood sample were collected and 4mls dispensed into vacutainer® plain tubes. After clotting, it was then centrifuged at 3000 g for 5 min. The serum was stored at -80°C until assayed. The remaining 2 ml were dispensed into fluoride oxalate tubes. Urine samples were assayed immediately for microalbuminuria and proteinuria using reagent test strips (DIRUI Co. Ltd,).

3.6 ANALYSIS

Glucose levels were estimated by the glucose oxidase method; albumin was also determined by the bromocresol green method, urea by the Urease- Berthelot method, creatinine by the modified Jaffe's method, electrolytes (sodium, potassium and chloride ions) were estimated by indirect potentiometry with ion selective electrodes, total calcium by the Arsenazo III method, urinary protein and microalbumin by the protein-error-of-indicator principle. Parathyroid hormone (PTH) was analyzed by EASIA using intact-hPTH EASIA kit from GenWay Biotech, Inc. (Catalogue number: 40-056-205022).

3.6.1 ***Biochemical assays:***

All serum and plasma biochemical assay were performed on Junior Flexor Chemical analyzer (Vital Scientific Co Ltd.). Parameters analyzed include albumin, glucose, urea, creatinine, total calcium, PTH and electrolytes (sodium, potassium and chloride). Adjusted calcium was calculated from the formula: Adjusted calcium (mmol/L) = total calcium (mmol/l) + 0.02 × [40 – serum albumin (g/dl)].

The methods adopted by the automated instrument for the determination of the above parameters are as follows and all reagents were from Vital Scientific Co Ltd.

3.7 PRINCIPLES UNDERLYING THE METHODS

3.7.1 ***Glucose***

Glucose is enzymatically oxidized by glucose oxidase to gluconic acid and hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide produces the oxidative coupling of phenol with 4-aminophenazone with a maximum absorbance at 500 nm according to the scheme:

$$\text{Glucose} + O_2 \xrightarrow{\text{Glucose Oxidase}} \text{Gluconic acid} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + \text{4-aminoantipyrine} \xrightarrow{\text{peroxidase}} \text{Quinoneimine} + 4H2O$$

3.7.2 ***Albumin (BCG)***

Principle and method

Albumin binds the dye bromocresol green (BCG) at a pH of 4.20 to form a complex with maximum absorption at 600 nm wavelength.

$$\text{Albumin} + \text{BCG} \xrightarrow{\text{pH} = 4.2} \text{Albumin-BCG complex}$$

3.7.3 *Creatinine*

Creatinine under alkaline conditions reacts with picrate ions forming a yellow-red complex which absorbs maximally at 600 nm. The formation rate of the complex is proportional to the concentration of the creatinine in the sample according to the equation below:

Creatinine + Picric acid $\xrightarrow{\text{Alkali (pH >12)}}$ Creatinine-picrate complex

3.7.4 *Urea*

Urea is hydrolyzed in the presence of water and urease to produce ammonia and carbon dioxide. The liberated ammonia reacts with α-ketoglutarate in the presence of NADH to yield glutamate. An equimolar quantity of NADH undergoes oxidation during the reaction catalyzed by Glutamate dehydrogenase (GLDH) resulting in a decrease in absorbance (340 nm) that is directly proportional to the urea nitrogen concentration in the sample.

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

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

3.7.5 *Calcium*

Calcium determination is based on the complexometric test (Arsenazo III). Arsenazo III 2,7-(bis(2-arsonophenylazo))-1,8dihydroxynaphtalene-3,6-disulphonic acid, forms in a neutral medium a blue complex with calcium. The colour intensity which absorbs maximally at 600 nm is directly proportional to the serum total calcium concentration.

Calcium + Arsenazo III $\xrightarrow{\text{Alkaline medium}}$ Calcium-Arsenazo Complex (Purple)

3.7.6 *Parathyroid Hormone Analysis*

3.7.6.1 *Clinical background and biological activity*

Human parathyroid hormone (hPTH) is a major physiological regulator of phosphocalcic metabolism. hPTH increases serum calcium concentrations by its actions on the kidney (enhancing tubular Ca^{2+} reabsorption and phosphate excretion) and bone (stmulating osteoclastic activity and bone resorption). It also indirectly affects intestinal absorption of Ca^{2+} by stimulating renal 1α-hydroxylation of 25-hydroxyvitamin D. The release of PTH is controlled in a negative feedback loop by the serum concentration of Ca^{2+}.

3.7.7 *Principle of the method*

The GenWay hPTH-EASIA is a solid phase Enzyme Amplified Sensitivity Immunoassay performed on microtitreplates. In this assay, calibrators and samples react with the capture polyclonal antibodies coated on microtitre well. After incubation, the excess of antigen is removed by washing. Then, monoclonal antibodies labeled with horseradish peroxidase (HRP) are added. After an incubation period, allowing the formation of a sandwich: coated PAbs - human PTH – Mab – HRP, the microtitre plate is washed to remove unbound enzyme labeled antibody. Bound enzyme-labelled antibody is measured through a chromogenic reaction. The chromogenic solution (TMB) is added and incubated. The reaction is stopped with the addition of a stop solution and microtitreplate is then read at the appropriate wavelength. The amount of substrate turnover is determined colourimetrically by measuring the absorbance, which is proportional to the PTH concentration in the sample.

Chapter 4

RESULTS

4.1 General Demographic and Clinical Characteristics of the Studied Population

The baseline biochemical and clinical characteristics of the studied population is as shown in Table 4.1. The mean age (51.6 ± 8.4 years) of the study participants was not significantly different from that of the control group (49.5 ± 10.6 years). Apart from waist to hip ratio, all the indicators of obesity showed significantly higher values when the case group were compared to the control group. Similarly, apart from serum calcium and its corrected form, as well as serum albumin and potassium which showed significantly lower values, all the other biochemical indicators (creatinine, urea, parathyroid hormone, sodium, proteinuria and microalbuminuria) showed significantly higher values among the case group when compared to the control group.

Table 4.1: General characteristics of the studied population

Parameters	Case Group (n = 200)	Controls (n = 100)	*p value*
Age (years)	51.6 ± 8.4	49.5 ± 10.6	0.3540
SBP (mm Hg)	187.9 ± 19.8	105.7 ± 9.8	< 0.0001
DBP (mm Hg)	104.5 ± 15.9	69.8 ± 7.6	< 0.0001
Height (m)	1.6 ± 0.1	1.6 ± 0.1	0.955
Weight (kg)	69.47 ± 12.9	63.5 ± 4.7	< 0.0001
BMI (kg m^{-2})	27.6 ± 4.9	25.4 ± 2.7	< 0.0001
Hip circumference (cm)	40.4 ± 4.4	35.3 ± 1.2	< 0.0001
Waist circumference (cm)	38.0 ± 4.4	33.2 ± 4.4	< 0.0001
Waist to hip ratio	0.9 ± 0.0	0.9 ± 0.0	0.712
Weight to height	43.8 ± 7.7	40.1 ± 3.2	< 0.0001
Albumin (g L^{-1})	42.5 ± 6.7	45.2 ± 3.4	0.0003
Creatnine (μmol L^{-1})	147.3 ± 119.6	70.4 ± 9.9	< 0.0001
Urea (mmol L^{-1})	5.3 ± 4.4	3.6 ± 0.7	0.0002
Calcium (mmol L^{-1})	2.2 ± 0.2	2.4 ± 0.1	< 0.0001
Corrected calcium (mmol L^{-1})	2.2 ± 0.2	2.3 ± 0.1	< 0.0001
Sodium (mmol L^{-1})	139.0 ± 4.8	137.9 ± 2.3	0.026
Potassium (mmol L^{-1})	3.9 ± 0.5	4.6 ± 4.2	0.022
Chloride (mmol L^{-1})	101.6 ± 4.2	100.1 ± 1.9	0.001
PTH	42.0 ± 35.2	22.4 ± 4.9	< 0.0001
Urine microalbumin			
Positive	81	0	
Negative	119	100	< 0.0001
Urine protein			
Positive	32	0	
Negative	168	100	< 0.0001

Data are presented as means ± SD and as categorical variables.p value defines the level of significance when case group was compared to controls (Unpaired t-test) and categorical variables for case group compared to controls (Chi-square test). SBP – systolic blood pressure; DBP – diastolic blood pressure; BMI – body mass index; PTH – parathyroid hormone

Figure 1 represents the distribution of case group by hypertensive class. The total percentage prevalence of the various forms of hypertension (mild, moderate, severe and very severe) increases from 1.0% to 10.5% and decreases to 8.5% through to 10.0% respectively with females having the highest prevalence of severe and the very severe form of hypertension whereas males dominate in the mild and moderate forms.

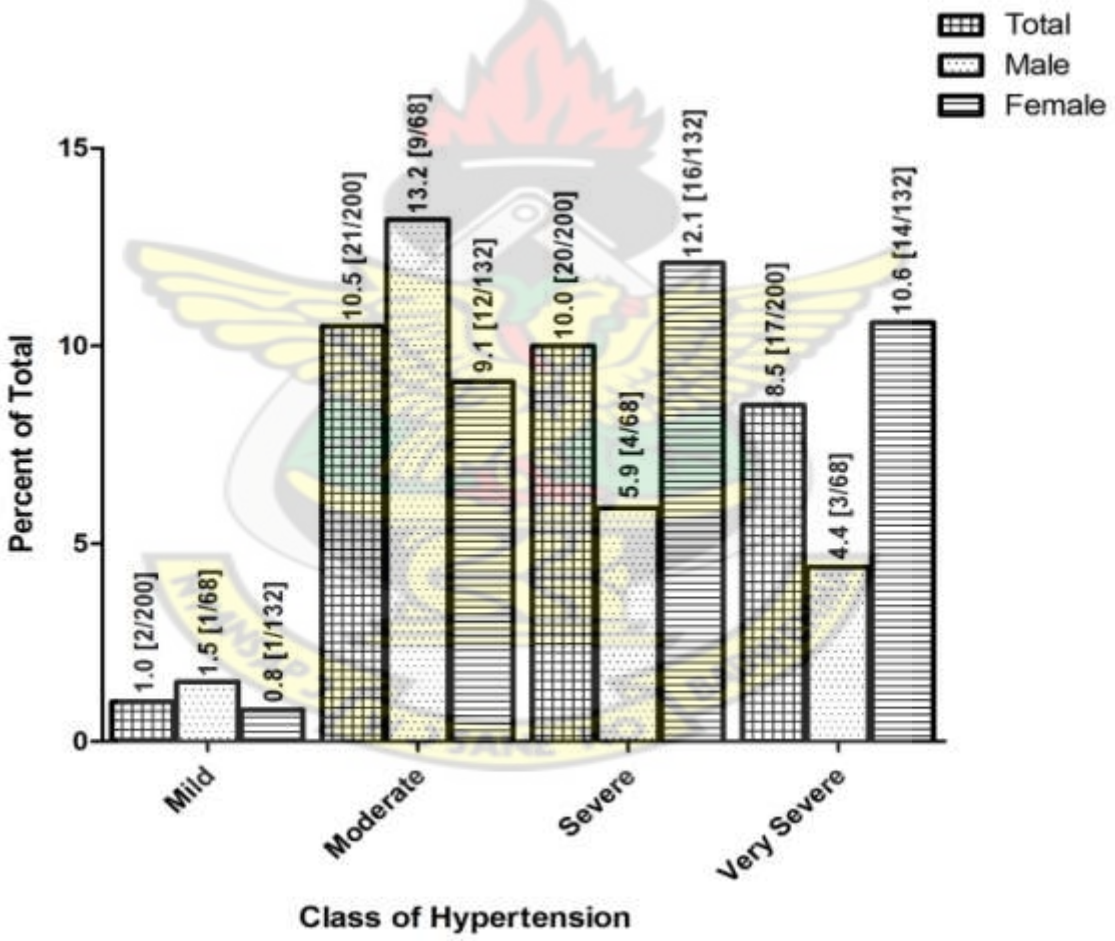

Figure 1: Distribution of case group by hypertensive class.

4.2 COMPARISON OF STUDY PARAMETERS BETWEEN CASE GROUPS AND CONTROLS STRATIFIED BY HYPERTENSION

From this table, the mean values of both systolic and diastolic blood pressures as well as the indices of obesity had significantly higher values and also increase as the severity of hypertension increases among the case group when subjects were compared to controls. However, there was no significant difference between subjects and controls in relation to height and waist to hip ratio. Again, the severity of hypertension increases as the subjects aged. When the subjects were stratified by hypertensive class, it was observed that creatinine and urea as well as parathyroid hormone (PTH) increases as the severity of hypertension increases with a concomitant decrease in both albumin and calcium.

Table 4.2: Comparison of study parameters between case groups and controls stratified by hypertension

Parameters	Control (n = 100)	HYPERTENSIVE CLASS				F	p value
		Mild (n = 2)	Moderate (n = 21)	Severe (n = 20)	Very Severe (n = 17)		
Age (years)	49.5 ± 10.6	57.5 ± 12.0	46.9 ± 7.2	53.8 ± 8.5***	55.7 ± 7.4***	11.6	< 0.0001
SBP (mm Hg)	105.7 ± 9.8	150.0 ± 0.0***	168.1 ± 4.0***	193.0 ± 8.0***	230.6 ± 19.8***	739.2	< 0.0001
DBP (mm Hg)	69.8 ± 7.6	92.5 ± 3.5**	100 ± 0.0***	110.3 ± 1.1***	139.4 ± 19.5***	298.3	< 0.0001
Height (m)	1.6 ± 0.1	1.6 ± 0.0	1.6 ± 0.1	1.6 ± 0.1	1.6 ± 0.0	0.908	0.461
Weight (kg)	63.5 ± 4.7	64.0 ± 5.7	67.9 ± 16.1	71.0 ± 14.7**	71.8 ± 8.3**	5.45	0.000
BMI (kg m^{-2})	25.4 ± 2.7	25.3 ± 1.8	27.5 ± 5.4	27.8 ± 5.7*	28.0 ± 3.5*	3.83	0.005
Hip circumference (cm)	35.3 ± 1.2	39.5 ± 0.7	40.4 ± 5.7***	41.3 ± 4.5***	41.7 ± 4.3***	32.01	< 0.0001
Waist circumference (cm)	33.2 ± 1.3	36.5 ± 0.7	38.0 ± 5.7***	39.0 ± 4.7***	39.2 ± 4.4***	28.26	< 0.0001
Waist to hip ratio	0.9 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	0.6638	0.618
Weight to height ratio	40.1 ± 3.2	40.2 ± 3.2	43.1 ± 9.2	44.4 ± 8.8**	44.8 ± 5.2**	5.027	0.001
Albumin (g L^{-1})	45.2 ± 3.4	45.3 ± 1.6	44.3 ± 5.4	42.1 ± 6.1*	36.4 ± 8.0***	13.29	< 0.0001
Creatinine (μmol L^{-1})	70.4 ± 9.9	86.4 ± 12.0	110.0 ± 57.4	197.2 ± 57.4***	367.0 ± 222.8***	46.11	< 0.0001
Urea (mmol L^{-1})	3.6 ± 0.7	4.0 ± 0.8	3.8 ± 0.9	4.3 ± 1.8	13.4 ± 9.6***	34.36	< 0.0001
Calcium (mmol L^{-1})	2.4 ± 0.1	2.4 ± 0.0	2.3 ± 0.2	2.2 ± 0.2***	1.9 ± 0.3***	37.68	< 0.0001
Sodium (mmol L^{-1})	137.9 ± 2.3	139.6 ± 13.3	138.5 ± 5.3	140.8 ± 7.1*	139.5 ± 5.6	2.372	0.055
Potassium (mmol L^{-1})	4.6 ± 4.2	4.1 ± 0.5	3.9 ± 0.5	4.0 ± 0.5	3.7 ± 0.6	0.4894	0.744
Chloride (mmol L^{-1})	100.1 ± 1.9	101.3 ± 1.2	102.5 ± 5.1*	102.4 ± 4.9*	100.8 ± 5.0	3.67	0.007
PTH (ng L^{-1})	22.4 ± 4.9	21.1 ± 1.9	28.4 ± 9.9	45.5 ± 24.1***	96.9 ± 68.2***	36.51	< 0.0001

Data are presented as means ± SD. *(p < 0.05), **(p < 0.001), ***(p < 0.0001) defines the levels of significance when hypertensive classes were compared with controls (One-way ANOVA with Dunnett's multiple comparison test); PTH – parathyroid hormone;SBP – systolic blood pressure; DBP – diastolic blood pressure; BMI – body mass index; NB: hypertensives who did not fall within the criteria used were not included in the above classification.

4.3 COMPARISON OF ESTIMATED GLOMERULAR FILTRATION RATES WITHIN THE STUDIED POPULATION STRATIFIED BY CKD STAGE.

Comparison of the estimated glomerular filtration rates (eGFR) within the studied population stratified by CKD stage as depicted in Table 4.3 indicates that the mean eGFR as determined by the various equations used (CG, CG-BSA, 4v-MDRD and CKD-EPI) were significantly lower among the study participants compared to the control group. Further, it depicts similar mean eGFR values among the subjects even though there were slight variations among the controls.When stratified by CKD stage, all the renal function equations interestingly generated similar mean values for subjects with moderate and severe CKD among the case group.

Table 4.3: Comparison of estimated glomerular filtration rates within the studied population stratified by CKD stage

	Equation	Mean eGFR	Stage 1 (≥ 90)	Stage 2 (60 – 89)	Stage 3 (30 – 59)	Stage 4 (15 – 29)	Stage 5 (<15)
CONTROLS	CG	97.2 ± 11.2	101.3 ± 7.9	81.1 ± 7.1	Nil	Nil	nil
	CG-BSA	102.1 ± 12.1	105.3 ± 9.4	82.2 ± 6.6	Nil	Nil	nil
	4V-MDRD	117.3 ± 14.1	117.6 ± 13.9	89.3 ± 0.0	Nil	Nil	nil
	CKD-EPI	115.8 ± 10.3	115.8 ± 10.3	Nil	Nil	Nil	nil
CASE GROUP	CG	62.0 ± 30.4***	113.2 ± 24.7***	74.8 ± 8.1**	46.6 ± 8.9	22.6 ± 5.3	10.9 ± 1.9
	CG-BSA	62.8 ± 29.0***	105.8 ± 16.4	74.1 ± 8.2***	46.1 ± 9.2	22.7 ± 4.9	11.4 ± 2.1
	4V-MDRD	67.1 ± 33.7***	110.9 ± 16.1**	72.8 ± 9.5	44.8 ± 9.1	21.9 ± 4.7	10.2 ± 2.4
	CKD-EPI	65.8 ± 33.0***	108.2 ± 11.4***	73.1 ± 9.0	45.9 ± 9.5	22.1 ± 5.2	9.1 ± 2.2

Data are presented as means ± SD. *p < 0.05, **p < 0.001, ***p < 0.0001 defines the level of significance when mean glomerular filtration rate for case group was compared to that of controls (Unpaired t-test); nil – no study participant fell with this group; CG – Cockcroft-Gault; CG-BSA – Cockcroft-Gault equation corrected for body surface area; CKD-EPI – Chronic Kidney Disease epidemiology equation; 4v-MDRD – four variable modification of diet in renal disease.

Table 4.4 demonstrates the prevalence of chronic kidney disease (CKD) among the study participants as per the estimating equations used. Even though there were variations in the mean percentage values with respect to mild CKD (stage 1), 4v-MDRD and CKD-EPI gave similar percentage values with CKD EPI and CG giving the highest and lowest percentage value respectively. For those with moderate CKD (stage 2 and 3), all the renal function equations used gave close percentage values apart from CG which gave the highest percentage prevalence of 36.0. CKD-EPI gave the lowest percentage prevalence of 26.5. Interestingly, all the renal function equations used generated similar percentage prevalence among study participants with severe CKD (stage 4 and 5) with 4v-MDRD and CKD-EPI giving similar percentage values. Furthermore, overall CKD prevalence among the studied population revealed similar percentage prevalence rates apart from CG which gave the highest overall prevalence rate of 50% with 4v- MDRD giving the lowest overall prevalence rate of 43.0%.

Table 4.4: Prevalence of chronic kidney disease among the study participants as determined with the estimating equations

Parameter	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	With CKD
Equation	**(≥ 90)**	**(60 – 89)**	**(30 – 59)**	**(15 – 29)**	**(<15)**	
			Control Group (n = 100)			
CG	80(80.0)	20(20.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
CG-BSA	86(86.0)	14(14.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
4V-MDRD	99(99.0)	1(1.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
CKD-EPI	100(100.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
			Case Group (n = 200)			
CG	28(14.0)	72(36.0)	69(34.5)	25(12.5)	6(3.0)	100(50.0)
CG-BSA	36(18.0)	69(34.5)	67(33.5)	20(10.0)	8(4.0)	95(47.5)
4V-MDRD	53(26.5)	61(30.5)	59(29.5)	16(8.0)	11(5.5)	86(43.0)
CKD-EPI	55(27.5)	53(26.5)	61(30.5)	20(10.0)	11(5.5)	92(46.0)

Data are presented as absolute values and proportions

4.5 STUDY VARIABLES AMONG HYPERTENSIVES STRATIFIED BY MICROALBUMINURIA

When the study population was stratified by microalbuminuria, it was observed that study participants with microalbuminuria had significantly higher levels of both systolic and diastolic blood pressures compared to normoalbuminuric subjects. Similary, significantly increased levels of serum creatinine, urea and PTH were observed among subjects with microalbuminuria compared to normoalbuminuric subjects. Serum albumin and calcium however, showed significantly lower values among study participants with microalbuminuria compared to normoalbuminuric subjects. Even though insignificant, all the indices of obesity with the exception of waist to hip ratio were higher among microalbuminuric subjects compared to their normoalbuminuric counterparts.

Table 4.5: Comparison of study variables among hypertensives stratified by microalbuminuria

Variables	Microalbuminuric (n = 81)	Normoalbuminuric (n = 119)	*p value*
Age (years)	54.5 ± 8.5	49.6 ± 7.8	< 0.0001
SBP (mm Hg)	200.1 ± 17.9	179.6 ± 16.6	< 0.0001
DBP (mm Hg)	114.3 ± 18.9	97.8 ± 8.6	< 0.0001
Height (m)	1.60 ± 0.1	1.58 ± 0.1	0.032
Weight (kg)	71.5 ± 12.6	68.1 ± 12.9	0.065
BMI (kg m^{-2})	28.0 ± 4.8	27.3 ± 5.0	0.343
Underweight	1(1.2)	2(1.7)	
Normal	21(25.9)	34(28.5)	
Overweight	38(46.9)	54(45.4)	
Obese	21(25.9)	29(24.4)	0.643
Hip circumference (cm)	41.4 ± 4.2	39.8 ± 4.5	0.011
Waist circumference (cm)	39.0 ± 4.3	37.3 ± 4.4	0.009
Waist to Hip ratio	0.9 ± 0.02	0.9 ± 0.02	0.295
Weight to Height ratio	44.7 ± 7.6	43.1 ± 7.8	0.150
Albumin (g L^{-1})	39.7 ± 8.3	44.5 ± 4.5	< 0.0001
FBS (mmol L^{-1})	5.2 ± 0.6	5.0 ± 0.6	0.122
Creatinine (μmol L^{-1})	229.8 ± 153.3	91.1 ± 18.7	< 0.0001
Urea (mmol L^{-1})	7.3 ± 6.1	3.9 ± 1.4	< 0.0001
Calcium (mmol L^{-1})	2.1 ± 0.3	2.3 ± 0.1	< 0.0001
Sodium (mmol L^{-1})	140.3 ± 5.9	138.1 ± 3.6	0.002
Potassium (mmol L^{-1})	4.0 ± 0.6	3.9 ± 0.4	0.112
Chloride (mmol L^{-1})	102.3 ± 5.2	101.1 ± 3.2	0.033
PTH (pg mL^{-1})	61.8 ± 48.1	28.6 ± 8.4	< 0.0001

Data are presented as means ± SD. p value defines the level of significance when microalbuminurics were compared with normoalbuminurics (Unpaired t-test); BMI – body mass index; FBS – fasting blood glucose; PTH – parathyroid hormone; SBP – systolic blood pressure; DBP – diastolic blood pressure

4.6 COMPARISON OF STUDY VARIABLES AMONG HYPERTENSIVES STRATIFIED BY PROTEINURIA

When the study variables among the hypertensives were stratified by proteinuria, it was observed that study participants with proteinuria had significantly higher mean values of both systolic and diastolic blood pressure compared to subjects without proteinuria. Biochemical parameters were also not different as albuminuric subjects had significantly increased levels of creatinine, urea and PTH with a corresponding decrease in serum albumin and calcium compared to subjects without albuminuria.

Table 4.6: Comparison of study variables among hypertensives stratified by proteinuria

Variables	Proteinuria (n = 32)	Absent Proteinuria (n = 168)	*p value*
Age (years)	55.2 ± 9.2	50.9 ± 8.1	0.0076
SBP (mm Hg)	211.9 ± 20.6	183.4 ± 16.1	< 0.0001
DBP (mm Hg)	125.3 ± 21.4	100.5 ± 10.9	< 0.0001
Height (m)	1.60 ± 0.1	1.58 ± 0.1	0.0747
Weight (kg)	71.7 ± 10.7	69.0 ± 13.3	0.2808
BMI (kg m^{-2})	27.9 ± 4.1	27.6 ± 5.0	0.7168
Underweight	0(0.0)	3(1.8)	
Normal	8(25.0)	47(28.0)	
Overweight	17(53.1)	75(44.6)	
Obese	7(21.9)	43(25.6)	0.8476
Hip circumference (cm)	41.6 ± 4.3	40.2 ± 4.4	0.0875
Waist Circumference (cm)	39.0 ± 4.7	37.8 ± 4.4	0.1546
Waist to Hip ratio	0.9 ± 0.03	0.9 ± 0.02	0.3343
Weight to Height ratio	44.7 ± 6.3	43.6 ± 8.0	0.4598
Albumin (g L^{-1})	35.8 ± 9.4	43.8 ± 5.2	< 0.0001
FBS (mmol L^{-1})	5.3 ± 0.6	5.0 ± 0.6	0.0495
Creatinine (mmol L^{-1})	342.0 ± 190.1	110.2 ± 41.1	< 0.0001
Urea (mmol L^{-1})	11.4 ± 8.1	4.1 ± 1.5	< 0.0001
Calcium (mmol L^{-1})	1.9 ± 0.3	2.3 ± 0.1	< 0.0001
Sodium (mmol L^{-1})	139.6 ± 5.7	138.9 ± 4.6	0.431
Potassium (mmol L^{-1})	4.1 ± 0.7	3.9 ± 0.4	0.1654
Chloride (mmol L^{-1})	101.8 ± 5.6	101.5 ± 3.8	0.7944
PTH (pg mL^{-1})	97.3 ± 53.5	31.5 ± 15.9	< 0.0001

Data are presented as means ± SD. p value defines the level of significance when microalbuminurics were compared with normoalbuminurics (Unpaired t-test); BMI – body mass index; FBS – fasting blood glucose; PTH – parathyroid hormone; SBP – systolic blood pressure; DBP – diastolic blood pressure

Figure 2 - 5 shows a linear regression analysis of various indices of renal dysfunction (creatinine, urea, sodium, potassium, calcium) and serum parathyroid hormone (PTH) in relation to systolic and diastolic blood pressures among study participants (A^1, B^1, C^1,) and controls (A, B, C). Every unit/mmHg increase in both SBP and DBP results in a significantly corresponding increase in the serum concentration of creatinine (p= <0.0001), urea (p= <0.0001), PTH (p= <0.0001) and Na^+ (p= 0.022 for SBP and p= 0.0014 for DBP) among the case group as compared to the controls. Furthermore, BP increases as the subjects aged. Conversely, there was a significant inverse relationship between calcium and both systolic and diastolic blood pressures with p-values of <0.0001 in each case. However, there was no linear regression between both SBP and DBP against potassium.

Fig. 6 - 7 also indicates a linear regression analysis of SBP and DBP against eGFR as estimated by the various renal function equations among the studied population. Significantly, there was an inverse relationship between systolic as well as diastolic blood pressures and all the renal function equations used (CG, CG-BSA, 4v- MDRD and CKD-EPI). Interestingly all the equations showed the same level of significance among the case group when analysed against both systolic and diastolic blood pressures (p= <0.0001) compared to the control group which generated insignificant correlations.

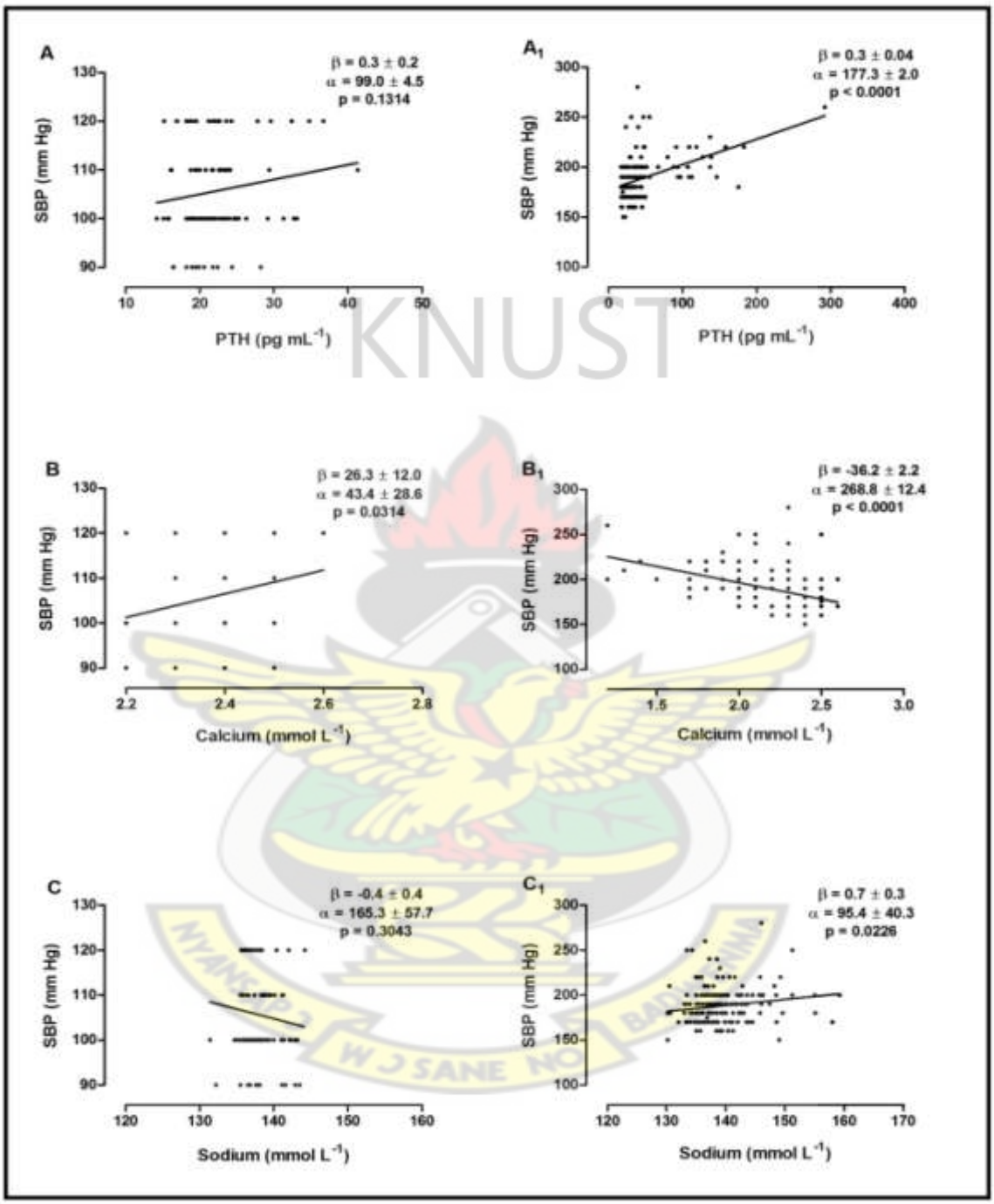

Figure 2: Regression analysis of systolic blood pressure against selected analytes in control subjects (A,B,C) and case groups (A_1,B_1,C_1)

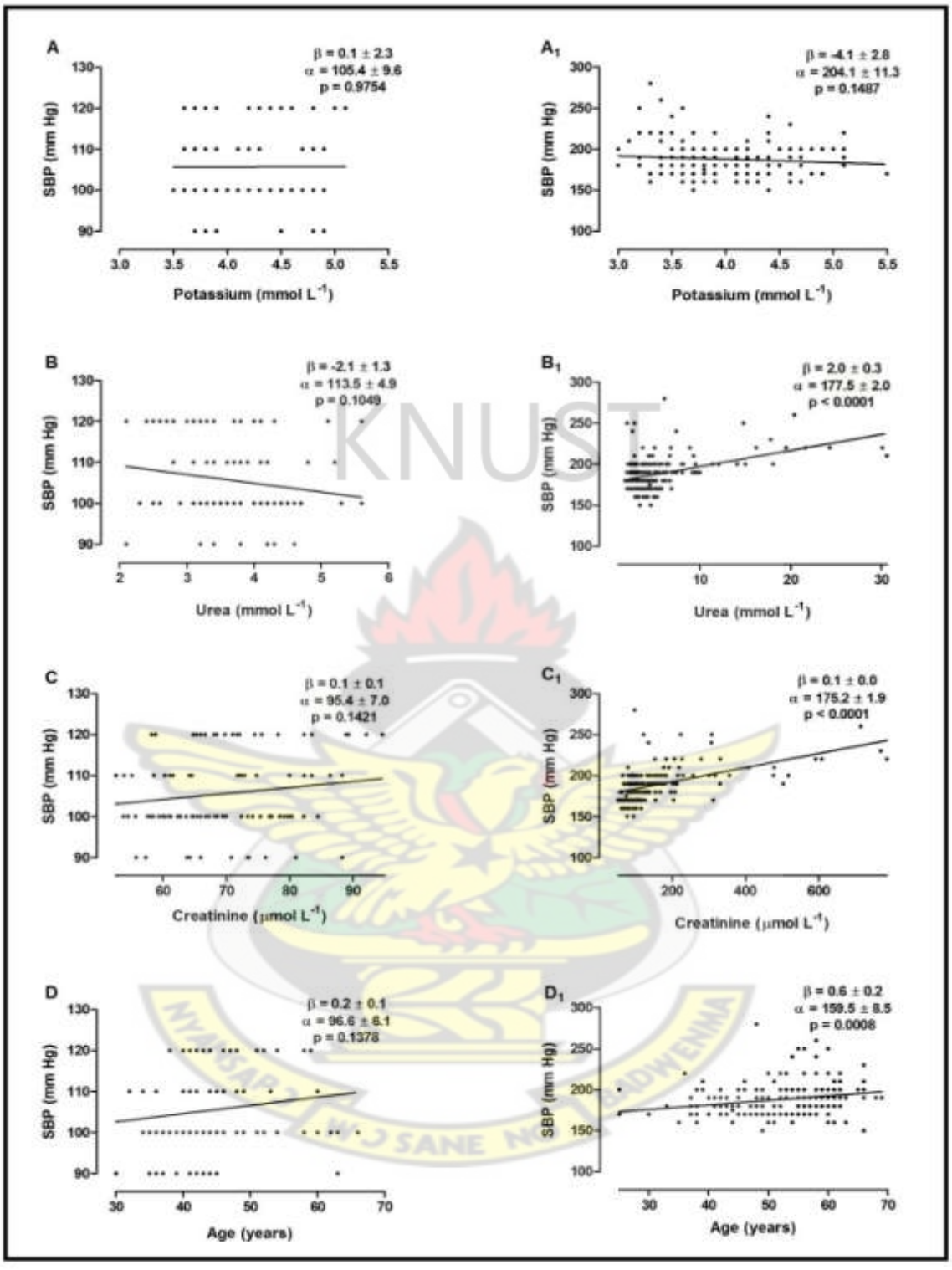

Figure 2: Regression analysis of systolic blood pressure against selected parameters in control subjects (A,B,C) and case groups (A_1,B_1,C_1

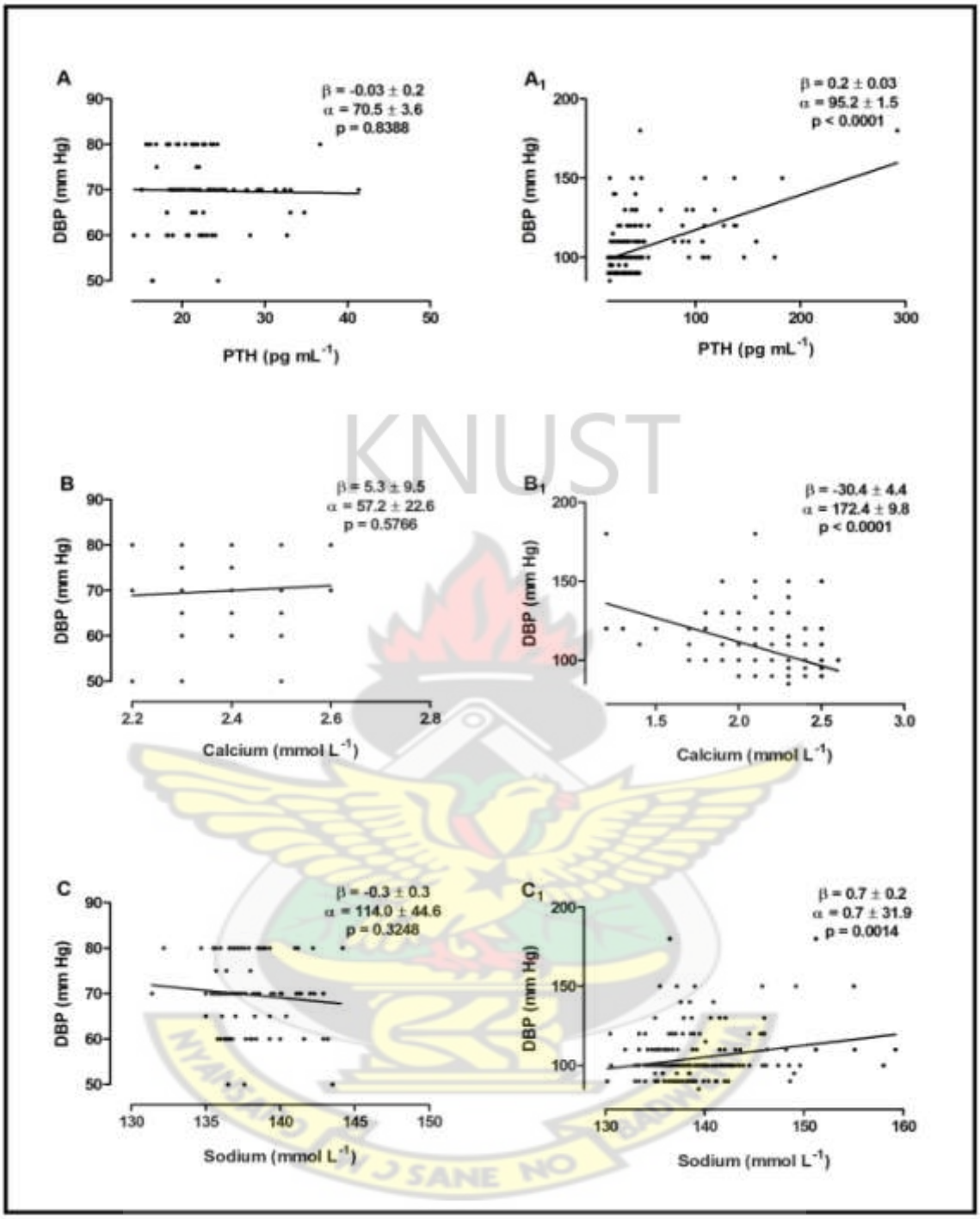

Figure 3: Regression analysis of diastolic blood pressure against selected analytes in control subjects (A,B,C) and case groups (A_1,B_1,C_1)

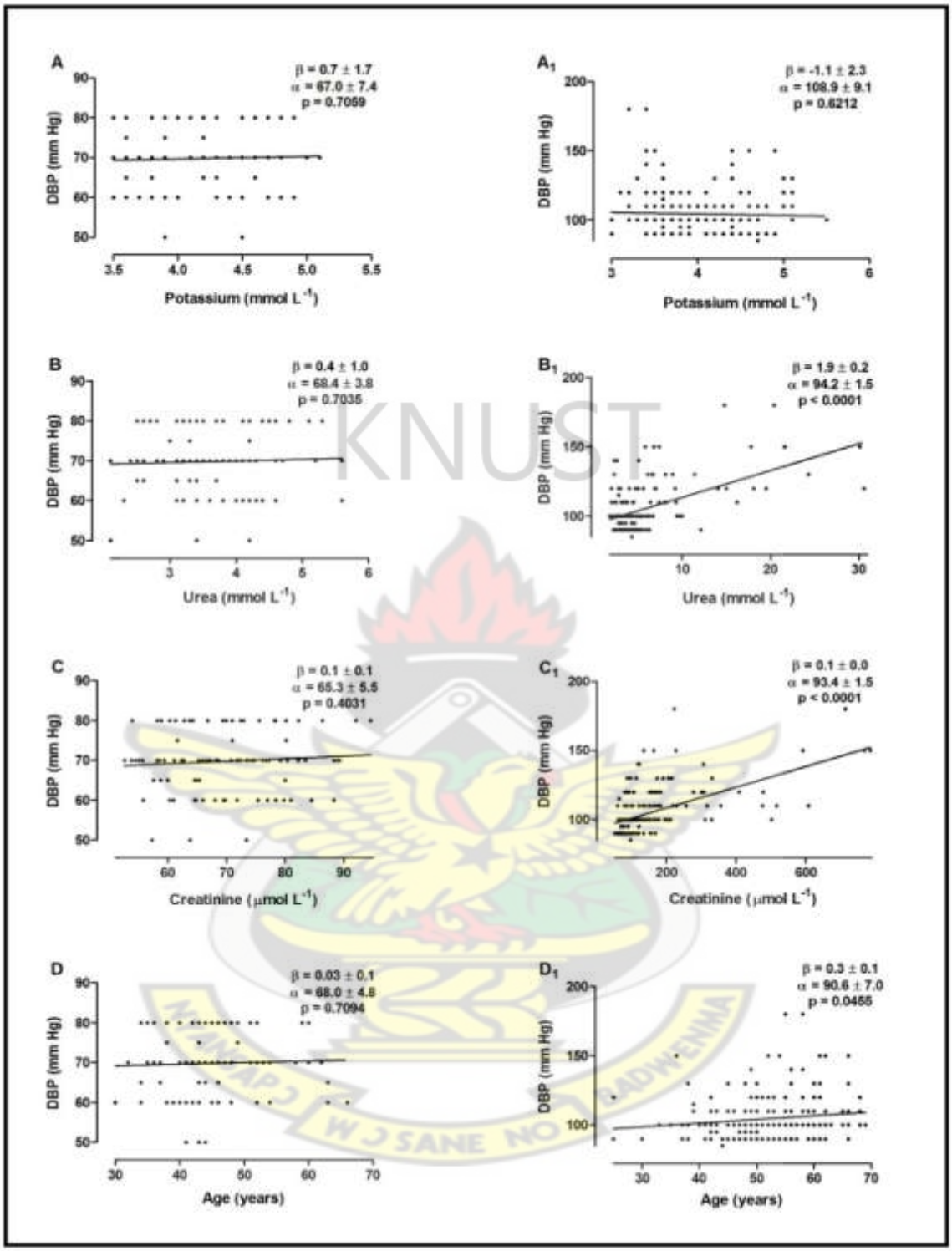

Figure 4: Regression analysis of diastolic blood against selected parameters in control subjects (A,B,C,D) and case groups (A_1,B_1,C_1,D_1)

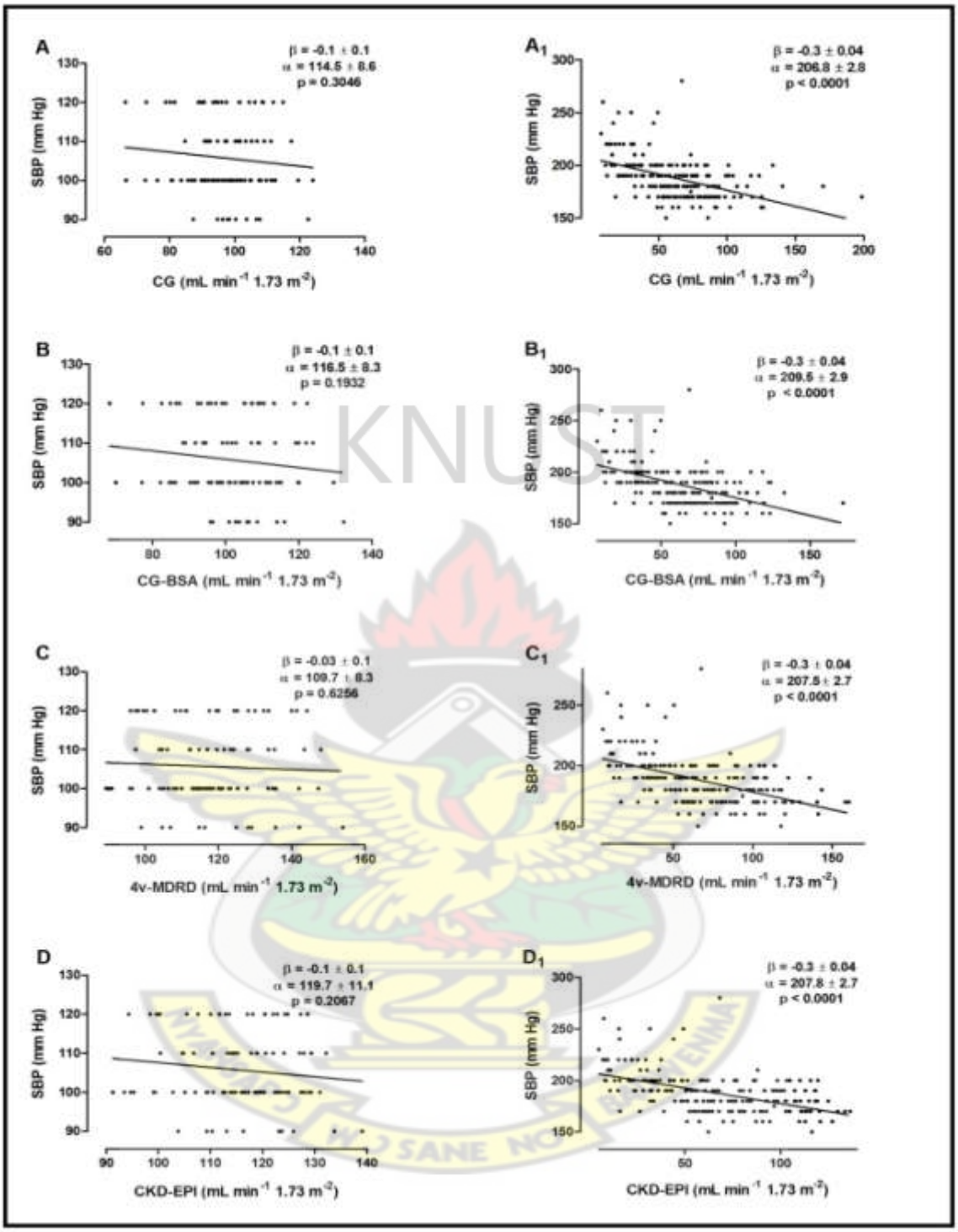

Figure 5: Regression analysis of systolic blood pressure against estimated glomerular filtration rate in control subjects (A,B,C,D) and case groups (A_1,B_1,C_1,D_1)

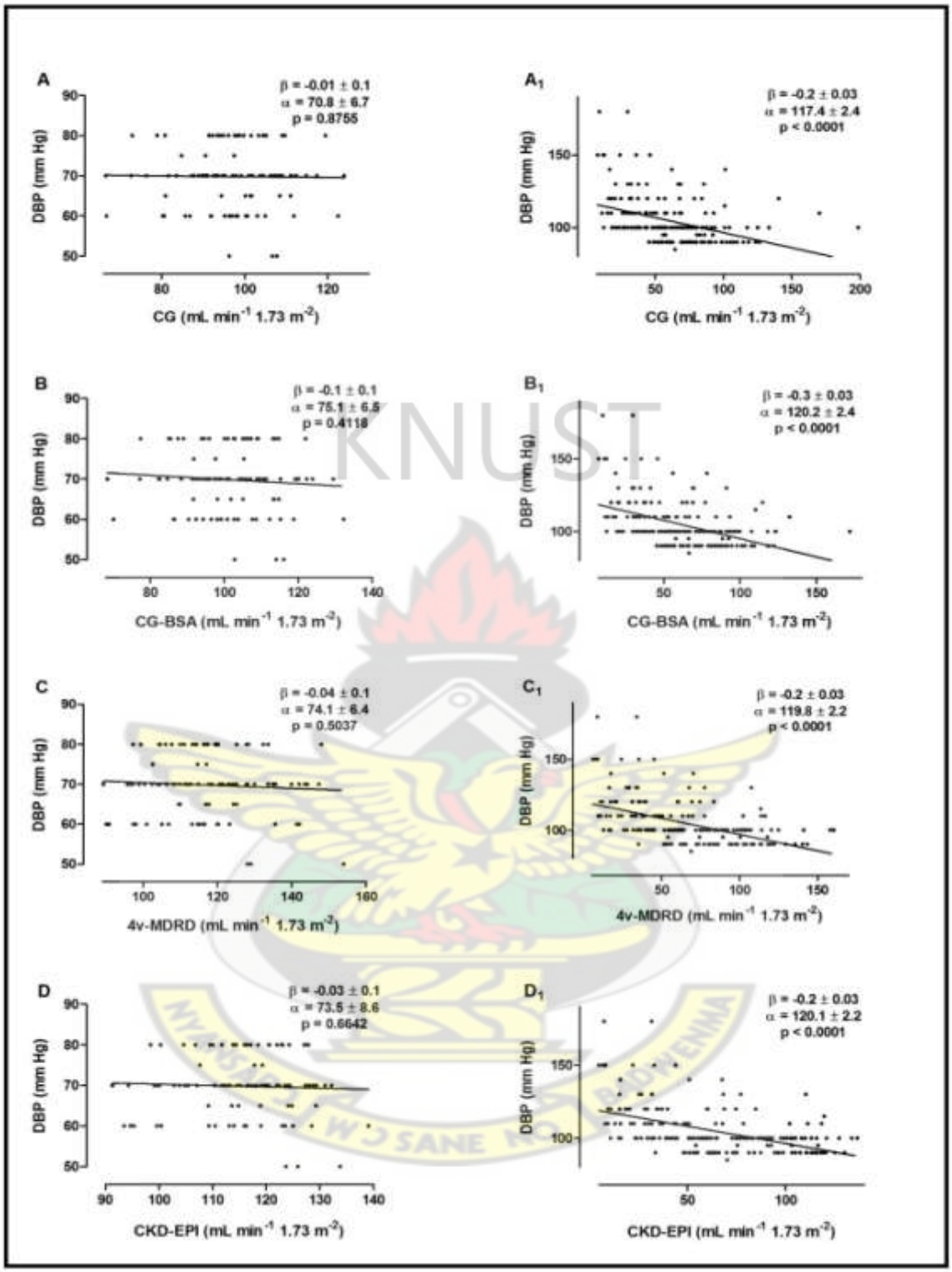

Figure 6: Regression analysis of diastolic blood pressure against estimated glomerular filtration rate in control subjects (A,B,C,D) and case groups (A_1,B_1,C_1,D_1)

CHAPTER 5

DISCUSSION

5.1 EXCRETION OF MICROALBUMINURIA AND ALBUMINURIA AMONG HYPERTENSIVES

The results from this study indicate a significant decrease in serum albumin among the hypertensives presenting with nephropathy with a commensurate increase in both microalbuminuria and albuminuria. This finding is in agreement with earlier work done by Jalal *et al.,* (2001), who reported a high prevalence of microalbuminuria as well as urinary albumin excretion rate with a decrease in serum albumin among hypertensives. Owiredu *et al.,* (2011) also reported a significant decrease in plasma albumin with a concomitant increase in proteinuria and hence kidney damage among individuals with CKD. Persistently increased proteinuria is mostly a marker of kidney damage. Excretion of albumin in large quantities has been identified as a sensitive marker for CKD attributable to hypertension and diabetes. Proteinuria implies that there is an increased excretion of albumin or some other identifiable protein in the urine Owiredu *et al.,* (2011). Excretion of microalbumin in the urine has recently been recognized as a predictive as well as an early marker for impending nephropathy and a more aggressive cardiovascular complication among hypertensives mainly because it indicates low but abnormal levels of albumin equivalent to 30mg/day which cannot be detected by the conventional proteinuric dip-sticks (Andronico, 1998, Jalal *et al.*, 2001). This presupposes that, it is not only an important marker for renal disease, but

above all, cardiovascular risk. Particularly, in hypertensives, microalbuminuria is associated with other signs of subclinical organ damage such as left ventricular hypertrophy, retinopathy and increased aortic stiffness (Giorami *et al.*, 2008). In addition to this, findings from this study indicates that apart from the significantly increased levels of creatinine and urea as well as the cardinal indices of obesity among the study population, it was also noted that these indicators significantly increases with the severity of hypertension which again emphasizes the need for early detection of renal dysfunction and control of hypertension.

As shown in table 4.1, there is a strong positive correlation (p-value <0.0001) between all the cardinal indices of obesity (BMI, WC, Wt., HC) and hypertension. Again, when the study population was stratified by hypertensive class, it was found out that obesity increases as the severity of hypertension increases among the study population. A finding similar to that reported by Sanjay and Tiware (2001). Obesity whether measured by increased BMI, WC or WHR have been shown to have a strong association not only with hypertension but also a number of cardiovascular risk factors such as diabetes mellitus, hyperlipidemia and even several cancers in many populations (Owiredu *et al.*, 2008). Indeed, central obesity, as evaluated by the waist circumference, has been indicated as an in dependent risk factor for renal dysfunction. Additionally, it has been demonstrated that increased body mass increases the risk for developing renal dysfunction (Owiredu *et al.*, 2011).

Several studies have shown that, in the past decade, obesity among Ghanaians has increased several times in most study populations (Owiredu *et al.*, 2008).

It is therefore not suprising that life threatening comorbidities especially hypertension and diabetes which are associated with obesity are on the ascendancy. Documented evidence indicates that hypertension is more common in obese individuals than non-obese individuals and further exhibit higher levels of plasma rennin activity, angiotensinogen and aldosterone as well as increased sodium retention (Vasilios *et al.*, 2010).

5.2 ELECTROLYTE PROFILES AMONG HYPERTENSIVES

In this study, the hypertensives had increased serum concentration of sodium and chloride but lower serum concentration of potassium compared to the controls. This is in conformity with findings among hypertensives in neighbouring Nigeria according to studies done by Iyalomhe *et al.,* (2008) who reported significantly higher levels of serum sodium and chloride with lower levels of potassium among essential hypertensives. This finding however differs from the findings of Adegoke *et al.,* (1990), who did not find any significant difference in these electrolytes between hypertensives and their normotensive counterparts. Sodium retention in the body is regulated by aldosterone; a mineralocorticoid hormone secreted through the activation of rennin-angiotensin system, with increased levels of aldosterone proportional to the sodium concentration and hence increased blood pressure (Owiredu *et al.,* 2011). There is considerable evidence that an inverse relationship exist between potassium and blood pressure, and that potassium has the ability to neutralize

the effect of sodium on blood pressure (Horacio and Medias, 2007). Thus, sodium and potassium has counter effect on blood pressure.

The fact that serum levels of sodium and chloride were significantly higher with lower serum potassium levels among the hypertensives compared to their normotensive counterparts may probably reflect increased potassium loss in exchanged for sodium load. It may also be due to derangements in the renin-angiotensin system, since aldosterone is a major determinant of potassium balance. Aldosterone stimulates potassium excretion both directly and indirectly (by increasing sodium reabsorption at the distal part of the tubules). Several properties of potassium including its antioxidant effect, its ability to suppress sympathetic nerve response to salt-sensitive hypertension, as well as its vasoactive effect which increases blood flow have all been found to reduce blood pressure and reduce risk to hypertension (Sanjay and Tiwari, 2001). Hence, increased sodium with lower potassium levels observed among the hypertensives confirms the important roles of these ions in the pathogenesis and maintenance of hypertension, and further affirms the rising trend of hypertensive cases in the various health care facilities across the country. Alternatively, this finding may imply a low intake of K^+ - rich diets including fresh fruits (eg. apples, bananas, orange, etc) and vegetables which has been demonstrated to lower blood pressure (especially among hypertensives than normotensives) (Iyalomhe *et al.*, 2008). In various human populations, K^+ has been identified as an important predictor of mean arterial pressure mainly

because hypertensives have been reported to have lower serum or plasma and total body K^+ levels than normotensives (Iyalomhe *et al.*, 2008).

5.3 PREVALENCE OF CKD AMONG THE STUDIED POPULATION

CKD is gradually becoming common in tropical Africa partly because risk factors such as hypertension and diabetes are on the ascendancy. Current guidelines emphasize the need to assess kidney function using predictive equations so as to optimize the care for individuals presenting with CKD (Owiredu *et al.*, 2011). In this study, the commonly used predictive equations in clinical practice (MDRD, CKD-EPI, CG, and CG-BSA) were applied to assess the prevalence of CKD among the study population. The results indicates that the prevalence of CKD among the hypertensives as indicated by MDRD, CKD-EPI, CG-BSA, CG were 43.0%, 46%, 47.5% and 50% respectively. This finding is consistent with a recent study by Osafo *et al.,* (2011) to identify the prevalence of CKD among hypertensives in Accra, Ghana in which the prevalence of CKD was found to be 46.9%. Available facts from the National Kidney Foundation (2002), indicates that several millions of people worldwide are at increased risk of developing CKD and that, hypertension and diabetes still remain the major risk factors accounting for up to two-thirds of all cases of CKD. These and other supporting facts including findings from this study presupposes that high BP is a common cause of CKD and hence patients with high BP are at increased risk of loss of kidney function and development of CKD. Individuals with high BP should therefore be carefully evaluated for the presence of CKD especially those with decreased GFR.

5.4 RELATIONSHIP BETWEEN PARATHYROID HORMONE (PTH), CALCIUM, BLOOD PRESSURE AND EGFR.

This study evaluated the relationships between PTH and calcium, PTH and blood pressure as well as eGFR and blood pressure. The significant inverse relationship between PTH and serum calcim among the hypertensive group compared to the controls, as well as the progressive increase in serum concentration of PTH as the severity of hypertension increases has been observed in earlier studies by Jorde *et al.*, (2000) who observed elevated serum PTH levels with significantly lower serum calcium among essential hypertensives. Young *et al.,* (1990) also reported similar findings among hypertensives.

This finding may suggest secondary hyperparathyroidism among these hypocalcaemic essential hypertensives. The main factor involved in the regulation of PTH secretion is the concentration of ionized calcium in the extracellular fluid, and a very important feature of the parathyroid gland is its sensitivity to small changes in serum calcium concentration (Locatelli *et al.*, 2002). Hence a reduction in ECF calcium concentration immediately leads to an increase in PTH secretion. Subsequently, it stimulates an increase in PTH synthesis and finally enhances parathyroid cell proliferation (Locatelli *et al.*, 2002). Hypocalcaemia therefore stimulates excess PTH secretion and may lead to secondary hyperparathyroidism due to hyperphosphataemia and decreased synthesis of 1,25-dihydroxycholecalciferol (calcitriol).

It is well known that the kidney may be both a cause and a victim of high blood pressure, and as to whether systolic or diastolic blood pressure poses a greater risk for renal function deterioration has become a matter of concern to most researchers. Other studies have reported that subjects with high mean diastolic blood pressure exhibited a significantly greater decline in renal function than subjects with lower diastolic pressure (Klahr, 1994; Brazy *et al.*, 1989) wheras others have also reported similar findings attributing decline in renal function to systolic blood pressure (Cerasola *et al.*, 2009).

In this study however, a strong positive association was observed between BP (both systolic and diastolic) and renal function deterioration among the hypertensives as per the estimated renal function equations used (MDRD, CKD-EPI, CG and CG-BSA). There was an equally significant positive association between age and both systolic and diastolic blood pressures, confirming a significant trend of increasing blood pressure with increasing age and renal function deterioration.

CHAPTER 6

CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

In conclusion, estimated GFR is moderately and severely reduced in a significant proportion of the studied population and that renal dysfunction is highly prevalent among non-diabetic hypertensives. The study has established that using the CKD-EPI, CG-BSA, MDRD and CG, chronic kidney disease is prevalent in about 46.0%, 47.5%, 43.0% and 50% respectively among the study population and that irrespective of the renal function equation used, the prevalence rate is high. In this study, 40.5% of the subjects presented with microalbuminuria where as 16% presented with proteinuria. In comparism however, none of the control subjects presented with either microalbuminuria or proteinuria. This finding therefore confirms the importance of microalbuminuria as an important marker of renal dysfunction. Further studies are however, warranted prior to the generalization of these findings for non-diabetic hypertensives. Health care institutions should institute measures for the early detection of renal dysfunction such as microalbuminuria to ameliorate the increasing prevalence of kidney dysfunction.

The results of the present study suggest that parathyroid hormone (PTH) is linked with derangements in the metabolism of calcium as well as the severity of hypertension and hence may contribute to the development of secondary hyperparathyroidism. PTH should therefore be measured early among

hypertensives especially those with the severe form of hypertension so as to be abreast with any changes and the necessary interventions provided to forestall the occurrence of any complication that may arise as a result of hyperparathyroidism**.** It is also imperative that appropriate public health education is initiated in all the districts of Ghana to advocate appropriate dietary habits and lifestyle in addition to exercising to promote good health among the general populase.

6.2 RECOMMENDATION

- Further studies are warranted in different populations among Ghanaian hypertensives to confirm the relationship between PTH and calcium as well as the severity of hypertension prior to its generalization.

- Microalbuminuria should be included as a routine tool as has been with proteinuria in the management of hypertensives in our various healthcare centres since it has been proven to be an early marker to pick distortions in renal disease.

- A study should be conducted to establish the role of renin and aldosterone in the aetiology of hypertensive nephropathy.

REFERENCES

Addo, J., Smeeth, L., Leon, DA (2009). Hypertensive target organ damage in Ghanaian civil servants with hypertension. *PLOS ONE* 4(8): 6672.

Adegoke, OA., Sofola, OA., Oditoyinbo, O (1990). Twenty four-hour urine sodium excretion and blood pressure in normotensive and hypertensive Nigerians in Lagos metropolis. *Niger. Med Practioner* 19: 1-2.

Agarwal, R., Anderson, MJ (2005). Correlates of systolic hypertension in patients with CKD. *Hypertension* 46: 514-520.

Aloia, JF., Fenerman, M., Yeh, JK (2006). Reference range for serum parathyroid hormone. *Endocr pract* 12(2): 137-144.

Amann, K., Gross, ML., London, GM., Ritz, E (1999). Hyperphosphatemia- a silent killer of patients with renal failure? *Nephrol Dial Transplant* 14: 2085-2087.

Amoah, GBA., Kallen, C (2000). Aetiology of heart failure as seen from the national cardiac referral centre in Africa. *Cardiology* 93 (1-2): 1-8.

Amoah, AG (2003). Hypertension in Ghana: a cross-sectional community prevalence study in Greater Accra. *Ethn Dis* 13(3): 310- 315.

Andronico, G., Ferrara, L., Mangano, M., Mule, G., Cerasola, G (1998). Insulin, sodium-lithium countertransport and microalbuminuria in hypertensive patients. *Hypertension* 31(1): 110-113.

Annual Report on Kwahu North District (2010). Kwahu North District Assembly. Unpublished.

Anthony, KK (1980). Patterns of cardiac failure in Northern Savanna Nigeria. *Trop georgr med* 32(2): 118-1125.

Ascherio, A., Hennekens, C., Willet, WC., Sacks, F., Rosner, B., Manson, J., Witheman, J., Meir, JS (1996). Prospective study of nutritional factors, blood pressure and hypertension among Us women. *Hypertension* 27: 1065-1072.

Bakris, G (2005). Proteinuria: A link to understanding changes in vascular compliance. *Hypertension* 46 (3): 473-474.

Barrett-Cornnor, E (1997). Sex difference in coronary heart disease. *Circulation* 95: 252-264.

Beamer, NB., Coull, BM., Clark, WM., Wynn, M (1999). Microalbuminuria in ischemic stroke. *Arch. Neurol* 56: 699-702.

Berton, G., Cordiano, R., Mbabo, S., De Toni, R., Mormino, P., Palatini, P (1998). Prognostic significance of hypertension and microalbuminuria for early mortality after acute myocardial infarction. *J. Hypertens* 6(9): 1325-1333.

Bolten, WK., Kliger, AS (2000). Chronic renal insufficiency: current understanding and implications. *Am. J. Kidney Disease* 36: 4-12.

Bonora, E., Targher, G., Zenere, MB., Saggiani, F., Cacciatori, V., Tosi, F., Travia, D., Zenti, MG., Branzi, P., Santi, L., Muggeo, M (1996). Relationship of uric acid concentration to cardiovascular risk factors in young men. Role of obesity and central fat distribution. The Verona young men artherosclerosis risk factors study. *Intern J Obesity* 20 (11): 975-980.

Bonsu, KW (2010). Epidemic of hypertension in Ghana: a systemic review. *BMC public Health* 10: 418.

Borzecki, AM., Kader, B., Berlowitz, DR (2009). The epidemiology and management of severe hypertension. *Journal of human hypertension* 24: 9-18.

Bouillon, R., Carmaliet, G., Boonen, S (1997). Ageing and calcium metabolism. *Clin endocrinol metab.* 11: 341-365.

Braam, B., Bluyssen, H., Kemmeren, P.,Holstege, F., Joles, JO., Koomans, H (2005). Nitric oxide dependent and nitric oxide independent transcriptional responses to high shear stress in endothelial cells. *Hypertension* 45: 672-680.

iBrazy, PC., Stead, WW., Fitzwilliams, JF (1989). Progression of renal insufficiency: role of blood pressure. Kidney int. 35: 670-674.

Breckenridge AM (1971). Prognosis of treated hypertension. *Br Heart J*. 33 (suppl): 127.

Burt, VL., Cutler, JA., Higgins, M., Horan, MJ., Labarthe, D., Whelton, P., Brown, C., Roccella, EJ (1995). Trends in the prevalence, awareness, treatment and control of hypertension in the adult US population: Data from the health examination surveys 1960-1991. *Hypertension* 26: 60-69.

Cannon, PJ., Statson, WB., Dimartini, FE., Sommers, SC., Laragh, JH (1996). Hyperuricaemia in primary and renal hypertension. *N. Engl J Med* 275: 457-464

Cappuccio, FP., Strazullo, P., Farinaro, E., Trevisan, M (1993). Uric acid metabolism and tubular sodium handling. *JAMA* 270: 354-359.

Cappuccio, FP., Micah, FB., Emmett, L., Kerry, MS., Antwi, S., Martin-Peprah, R., Phillips,OR., Plange-Rhule, J., Eastwood, JB (2004). Prevalence, detection, management and control of hypertension in Ashanti, West Africa. *Hypertension* 43: 1017-1022.

Cerasola, G., Mule, EN., Cusimano, P., Palermo, A., Arsena, R., Guarneri, M., Geraci, C., Cottone, S (2010). Clinical correlates of renal dysfunction in hypertensive patients without cardiovascular complications. The REDHY study. *Journal of human hypertension* 24: 44-50.

Cerasola, G., Mule, G., Nardi, E., Cusimano, P., Palermo, A., Arsena, R., Guarneri, M., Geraci, C., Cotton, S (2009). Clinical correlates of renal dysfunction in hypertensive patients without cardiovascular complications. The REDHY study. *Journal of human hypertension* 24: 44-50.

Chaban, JS., Brizanti, ME., Kerr, PG., Dustan, DW., Welborn, TA., Zimmet, PZ., Atkins (2003). Prevalence of kidney damage in Australian adults. *J Am. Soc. Nephrol* 14: S131-S138.

Cirillo, M., Laurenzi, M., Trevisan, M., Stamler, J (1992). Haematocrit, blood pressure and hypertension. the Gubbio population study. *Hypertension* 20: 319-326.

Clark, M and Kumar, P (2005). Clinical medicine. 6th ed. Elsevier Saunders. Pp 857-859.

Cocoran, AC., Taylor, RD., Page, IF (1948). Functional patterns in renal disease. *Ann Intern Med.* 28: 560-582.

Cohuet, G., Struijker-Bondier, H (2006). Mechanism of target organ damage caused by hypertension: therapeutic potential. *Pharmacology and therapeutics* 3(1): 81-98.

Connel, JMC., Jamieson, A., Davies, E., Ingram, MC., Soro, A., Frazer, R (1999). 11 beta-hydroxylase activity in glucocorticoid suppressible hyperaldosteronism: Lessons for essential hypertension? *Endocri Res*. 22: 691-700.

Cooper, RS., Amoah, AG., Mensah, GA (2003). High blood pressure: the foundation for epidemic cardiovascular disease in African populations. *Ethn Dis*. 13 (2 Suppl 2): S48-S52.

Coresh, J., Wei, GL., Mc Quillan, G., Brancati, FL., Levey, AS., Jones, C., Klaq, MG (2001). Prevalence of high blood pressure and elevated serum creatinine level in the US. *Arch intern med*. 161: 1207-1216.

Sierra, A., Bragulat, E., Gomez-Angelats, E., Antonio MT., Aquilera, MT., Coca, A (2000). Microalbuminuria in essential hypertension: clinical and biochemical profile. *Br J Biomed Sci* 57 (4): 287-291.

de Simone, G., Tommaselli, AP., Rossi, R., Valentino, R., Scopacasa, F., Lombardi, G (1985). Partial deficiency of adrenal 11 beta-hydroxylase: A possible cause of essential hypertension. *Hypertension* 7: 613-618.

Drueke, TB (1995). The pathogenesis of parathyroid gland hyperplasia in chronic renal failure. *Kidney int*. 48: 259-272.

Drueke, TB (2000). Cell biology of parathyroid gland hyperplasia in chronic renal failure. *J Am Soc Nephrol* 11: 1141-1152.

du Cailar, G., Ribstein, J., Mimran, A (2002). Diatary sodium and target organ damage in essential hypertension. *Am J Hypertens* 15: 222-229.

Dustan, HP., Schneckloth, RE., Corcoran, AC., Page, RI (1958). The effectiveness and long-term treatment of malignant hypertension. *Circulation* 18: 644.

Epstein, M., Oster, JR (1984). Hypertension. A practical approach. 1st ed. WB Saunders company, UK. Pp. 3-45.

Facchini, F., Hollenbeck, CB., Chen, YN., Chen, YD., Reaven, GM (1992). Demonstration of relationship between white blood cell count, insulin resistance and several risk factors for coronary heart disease in women. *J intern med* 232: 267-272.

Fishberg, AM (1927). The arteriolar lesions of glomerulonephritis. *Arch intern med*. 40: 80.

Flack, JM., Poters, R., Shafi, T.,Alrefai, H., Samar, AN., Crooke, E (2002). Prevention of hypertension and its complications: theoretical basis and guidelines for treatment. *J Am Soc Nephrol* 14: S92-S98.

Fogo, A., Breyer, JA., Smith, MC., Cleveland, WH., Agodoa, L., Kiek, KA., Glassock, R (1997). Accuracy of the diagnosis of hypertensive nephrosclerosis in African-Americans. A report from the African-American study of kidney disease trial. *Kidney int*. 51: 244-252.

Ford, ES (2003).C-reactive protein concentration and cardiovascular disease risk factors in children: findings from the National Health and Nutrition Examination survey. *Circulation* 108: 1053-1058.

Francisco, L (1995). Secondary hyperparathyroidism in renal failure. *American Journal of Kidney diseases* 25(5): 663-679.

Freedman, BI., Sedor, JR (2008). Hypertension associated kidney disease: perhaps no more. *J Am Soc Nephrol* 19: 2047-2051.

Friedman, GD., Selby, JV., Quensenberry, CP (1990). The leukocyte count: A predictor of hypertension. *J clin epidemiol* 43:907- 911.

Fukuda, N., Tanaka, H., Tominaga, Y., Fukagawa, M., Kurokawa, K., Seino, Y (1993). Decreased 1,25- dihydroxy-vit.D3 receptor is associated with a more severe form of parathyroid hyperplasia in chronic uremic patients. *J clin invest* 92: 1436-1443.

Genest, J., Lemieux, G., Davidnon, A., Koiw, E., Nowaczynski, W., Steyermark, P (1956). Human arterial hypertension: a state of mild chronic hyperaldosteronism? *Science* 123: 503.

Gilber, RP., Kurth, T., Kausz, AT., Manson, JE., Buring, JE (2005). Association between CKD and body mass index in apparently healthy men. Am. J Kidney Dis. 46: 871.

Gillum, RF (1996). The epidemiology of cardiovascular disease in black Americans. *N. Engl. J. Med.* 335: 1597-1599.

Giorami, C., Mule, G., Santina, C., Emilio, N., Paola, C (2008). Hypertension, microalbuminuria and renal dysfunction study. *J. Nephrol* 21: 368-373.

Goodman, WG., Goldin, J., Knizon, BD., Yoon, C., Gales, B (2000). Coronary artery calcification in young adults with end-stage renal disease who are undergoing dialysis. *N Engl J med* 342: 1478-1483.

Griffin, KA (2006). Hypertension and kidney damage. *J Clin Hypertens* 8(3): 209-214.

Gruden, G., Gnudi, L., Viberti, G (2004). Pathogenesis of diabetic nephropathy: fundamental and clinical text. 3rd ed. Lippincott Williams and Wilkins publishers, Philadelphia. Pp 1315-1330.

Honda, M., Nowaczynsci, W., Guthrie, G., Mersserli, F., Torlis, G., Kuchel, O., Genest, J (1975). Response of several adrenal steroids to ACTH stimulation in essential hypertension. *J clin endocrinol metab*. 44: 264-272.

Horacio, JA., Madias, EN (2007). Sodium and potassium in the pathogenesis of hypertension. *The New England journal of medicine* 356(19): 1966-1978.

International society of hypertension guidelines for the management of hypertension-WHO (1999). *J hypertens* 17: 151-183.

Iseki, K., Ikemiya, Y., Fuliyama, K (1997). Risk factors for end-stage renal disease and serum creatinine in a community-based mass screening. *Kidney int*. 51: 850-854.

Isezno, AS., Omotoso, ABO., Gaye, A., Corrah, T., Araoye, MA (2000). One year survival among sub-saharan Africans with hypertensive heart failure. *Tropical cardiology* 26(103): 57-60.

Isles, CG., Hole, DJ., Howthorn, VM., Lever, AF (1992). Relationship between coronary risk and coronary mortality in women: comparism with men. *Lancet* 339: 702-706.

Iyalomhe, GBS., Omogbai, EKI., Ozula, IR., Folorunso, LD., Osigbemhe, OBJ (2008). Electrolyte profiles in Nigerian patients with essential hypertension. *African Journal of Biotechnology* 7 (10): 1404-1408.

Jacques, G., Otto, K., Hamet, P., Marc, C (1983). Hypertension, physiopathology and treatment. 2nd ed. McGraw-Hill book company, New York. Pp 198-209.

Jalal, S., Sofi, FA., Alai, MS., Sadiqqui, MA., Bhat, MA., Khan, KA., Jan, VM., Lone, NA., Rather, HA (2001). Prevalence of microalbuminuria in essential hypertension: A study of patients with mild to moderate hypertension. *Indian Journal of nephrology* 11: 6-11.

James, MA., Fortherby, MD., Potter, JF (1995). Screening test for microalbuminuria in non-diabetic elderly subjects and their relation to blood pressure. *Clin. Sci*. 88:185-190.

Jansson, JH., Johanson, B., Boman, K., Nilsson, TK (1991). Hypofibrinolysis in patients with hypertension and elevated cholesterol. *J Intern Med* 229: 309-316.

Jorde, R., Sundsfjord, J., Hang, E., Bonaa, KH (2000). Relation between low calcium intake parathyroid hormone and blood pressure. *Hypertension* 35(5): 1154-1159.

Kannel, WB., Castelli, WP., McNamara PM., Mckee, PA., Feinleib, M (1972). Role of blood pressure in the development of congestive heart failure. The Framingham study. *New England Journal of Med*. 287: 781.

Kannel, WB., Zhang, T., Garrison, RJ (1992). Is obesity related hypertension less of a cardiovascular risk? The Framingham study. *Am Heart J* 120: 1195-1201.

Kaplan, NM (2006). Primary hypertension: Pathogenesis. 9th ed. Lippincott Williams and Wilkins publishers, Philadelphia. Pp. 50-120.

Kappanen, H (1991). Minerals and blood pressure. *Ann Med* 23(3): 299-305.

Kendal, MJ., Kaplan, MN., Hoton, CR (1995). Difficult hypertension, practical management and decision making. 1st ed. Martin Dunitz Ltd. UK, London.

Kengne, PA., Awal, PK., Fezen, L., Mbanya, JC (2007). The burden of high blood pressure and related risk factorsin urban sub-saharan Africa: evidence from Douala in Cameroon. *Afr Health Sci* 7(1): 38-44.

Kirkendall W.M., Burton A.C., Epstein F.H. and Freis E.D. (1967) Recommendations for human blood pressure determination by sphygmomanometers. *Circulation* 36, 980-988.
Klabunde, RE (2005). Cardiovascular physiology concepts. 5th ed. Lippincott Williams and Wilkins. Pp 385-391.

Klag, MJ., Whelton, PK., Coresh, J., Grim, CE., Kuller, LH (1995). The association of skin colour with blood pressure among US blacks with low socioeconomic status. *JAMA* 265: 599-602.

Klahr, S., Levey, AS., Caggiula, AW., Beck, GJ., Hunsicker, L., Kusek, JW., Striker, G (1994). The effect of dietary restrictions and blood pressure control on the progression of chronic kidney disease. *N. Engl. J. Med* 330: 877-874.

Komiya, I., Yamada, T., Aizawa, T., Takasu, N., Maruyama, Y., Ogawa, A (1991). Inappropriate elevation of the aldosterone/plasma rennin activity ratio in hypertensive patients with increases of 11-deoxycorticosterone and 18-hydroxy-11-deoxycosterone: a subtype of essential hypertension? *Cardiology* 78: 99-100.

Korner, PI (2007). Essential hypertension and its causes: neural and non-neural mechanisms. Oxford Univesity press. Pp 341-348.

Ladipo, GO., Fronde, JR., Parry, EH (1997). Patterns of heart disease in adults of Nigerian Savanna: A prospective clinical Study. *Afr J Med Sci* 6(4): 185-192.

Landin, K., Tengborn, L., Smith, U (1990). Elevated fibrinogen and plasminogen activator inhibitor (PAI-1) in hypertension are related to metabolic risk factors for cardiovascular disease. *J intern med* 27: 273-278.

Levy, AS., Coresh, J,. Balk, E., Kausz, AT., Levin, A., Steffes, MW., Hoqq, RJ., Perrone, RD., Lau, J., Eknoyan, G (2003). National Kidney Foundation practical Guidelines for CKD: elevation classification and stratification. *Ann intern med*. 139(2): 137-147.

Lifton, RP., Dluhy, RG., Powers, M., Rich, GM., Gutkin, M., Fallo, F., Gill, JR., Feld, L., Ganguly, A., Laidlaw, JC (1992). A chimaeric 11 beta-hydroxylase/aldosterone synthase gene causes glucorticoid-remediable aldosteronism and human hypertension. *Nature* 355: 262-265.

Lim, PO., Rodjers, P., Cardale, K., Watson, AD., Macdonald, TM (1999). Potentially high prevalence of primary aldosteronism in a primary care population. *Lancet* 353-340.

Lionel, H., Yackoob, KS (2005). Hypertension in sub-saharan African populations. *Circulation* 112: 3562-3568.

Llach, F (1995). Secondary hyperparathyroidism in renal failure: the trade-off hypothesis revisited. *American journal of kidney diseases* 25(5): 663-679.

Locatelli, F., Cannata-Andia, JB., Tilman, BD., Walter, HH., Forque, D., Olof, H., Ritz, E (2002). Management of disturbance of calcium and phosphorus metabolism in chronic renal insufficiency, with emphasis on the control of hyperphosphatemia. *Nephrol Dial Transplant* 17(5): 723-731.

Luft, CF (1997). Microalbuminuria and essential hypertension: renal and cardiovascular implications. *Journal of hypertension* 6(6): 134-142.

MacMahon, S., Peto, R., Collins, R (1990). Blood pressure, stroke and coronary artery disease: overview of randomized drug trials in their epidemiological context. *Lancet* 335: 827-839.

Mariano, R., Sagrario, C. Ignacio, L., Aguilera-Tejero, E., Yolanda, A (2006). Regulation of parathyroid function in chronic kidney failure. *Journal of bone and mineral metabolism* 24(2): 164-168.

Martins, D., Tareen, N., Norris, KK (2002). The epidemiology of end-stage renal disease among African-Americans. *Am J Med Sci* 323: 65-71.

Mitch W.E. (2007) Chronic Kidney Disease, 23rd ed. Philadelphia, Pa,: Saunders Elsevier.

National Kidney Foundation (2002). K/DOQI Clinical Practice Guidelines for Chronic Kidney Disease: Evaluation, Classification and Stratification. *Am J Kidney Dis* 39: S1 - S266 (supp 1).

Neaton, JO., Wentworth, D., Sherwin, R., Kittner, S., Kuller, L., Stamler, J (1989). Comparism of 10 year coronary and cerebrovascular disease mortality rates by hypertensive status for black and non-black men screened in the Multiple Risk Factor Intervension Trial (MRFIT). *Circulation* 80: 300.

Norman, KM (1994). Clinical hypertension. 7th ed. Williams and Wilkins, UK. Pp 115-119.

Onovughakpo-Sakpa, O., Oyeneke, EC., Olumese, EF (2009). Incidence of diabetic nephropathy in Southern Nigeria. *J. Med. Sci*. 9(6): 264-269.

Onwuchekwa, AC., Asekome, GE (2009). Pattern of heart failure in a Nigerian Teaching Hospital. Health risk management 5: 745-750.

Osafo, C., Mate-Kole, M., Affram, K., Adu, D (2011). Prevalence of Chronic Kidney Disease in hypertensive patients in Ghana. *Ren Fail* 33(4): 388-392.

Owiredu, W., Adamu, M., Amidu, N., Woode, E., Bam, V., Plange-Rhule, J., Opoku-Okrah, C (2008). Obesity and cardiovascular risk factors in a Penticostal populationin Kumasi, Ghana. *J. Med. Sci*. 8: 682-690.

Owiredu, WKBA., Dadzi, ER., Amidu, N., Eghan, BA., Quaye, L (2011). Predictive equations, oxidative and metabolic risk factors among Ghanaian patients presenting with chronic kidney disease. *In press*.

Owusu, IK (2007). Causes of heart failure as seen in Kumasi. *Journal of third world medicine* 5(1): 1-8.

Psaty, BM., Siscovick, DS., Weiss, NS., Koepsell, TD., Rosendaal, RF., Danyu, L., Susan, RH., Wagner, EH., Curt, DF (1996). Hypertension and outcomes research. J hypertens 9: 178-183.

Pedrinelli, R., Dell'Omo, G., Di Bello, V., Pontrimoli, R., Mariani, M (2002). Microalbuminuria, an integrated marker of CV risk in essential hypertension. *Journal of human hypertension* 16: 79-89.

Perera, GA (1995). Hypertensive vascular disease: Description and natural history. *J chronic dis* 1: 33-42.

Peronne, RD., Madias, NE., Levey, AS (1992). Serum creatinine as an index of renal function: new insights into old concepts. *Clin chem*. 38: 1933-1953.

Pfeifer, M., Begerow, B., Helmut, WM., Detlef, N., Hasen, C (2001). Effects of a short term calcium and vitamin D3 supplementation on blood pressure and parathyroid hormone levels in the elderly. *Journal of clinical endocrinology and metabolism* 86(4): 1633-1637.

Pitts, TO., Piraino, BH., Mitro, R., Chem, TC., Segre, GV., Greenberg, A., Puschette, JB (1988). Hyperparathyroidism and 1,25-dihydroxyvitamin D deficiency in mild, moderate and severe renal failure. *J. Clin Endocrinol Metab* 67: 876-881.

Plange-Rhule, J., Philips, R., Acheampong, JW., Sagger-Malik, AK., Cappuccio, FP., Eastwood, JB (1999). Hypertension and renal failure in Kumasi, Ghana. *Journal Hum Hypertens* 13(1): 37-40.

Pontremoli, R (1996). Microalbuminuria in essential hypertension- a relation to cardiovascular risk factors. *Nephrol Dial Transplant* 1: 2115-2116.

Pontremoli, R., Nicolell, C., Viazzi, F., Ravera, M., Sotia, M., Berruti, V., Benzente, GP., Del, SM., Martinoli, C., Sacchi, G., Deferrari, G (1998). Microalbuminuria is an early marker of target organ damage in essential hypertension. *Am J Hypertens* 11: 430-438.

Resnick, LM., Muller, FB., Laragh, JH (1986). Calcium regulating hormone in essential hypertension: relation to plasma rennin activity and sodium metabolism. *Ann intern med.* 105: 649-654.

Rolf, J., Johan, S., Egil, H., Kaare, HB (2000). Relation between low calcium intake, parathyroid hormone and blood pressure. *Hypertension* 35: 1154-1159.
Rosa, T T and Palatini, P (2000). Clinical value of microalbuminuria in hypertension. *J. Hypertens* 18(6): 645-654.

Rosa, TT., Palatini, P (2000). Clinical value of microalbuminuria in hypertension. *J. Hypertens* 18(6): 645-654.

Sanjay, V., Tiwari, SC (2001). Essential hypertension – pathogenesis and pathophysiology. Jounal, Indian Academy of Clinical Medicine 2(3):140-150.

Saydah, S., Rios-Burrows, N., Williams, D., Geiss, L., Dorsey, S., Eberhardt, M (2007). Prevalence of CKD and associated risk factors in the US, 1999-2004. *CDC* 56(8): 161-165.

Schoolwerth, AC., Engelgan, MM., Hostetter, TH., Kathy, HR., Chianchiano D., McClellan, WM., Warnock, DG., Vinicor, F (2006). Chronic Kidney disease: A public health problem that needs a public health action plan. *Prev chronic dis.* 3: A57.

Seema, B., Pierre, F., Mimran, A., Julia, BL (2008). Microalbuminuria in hypertension and type 2 diabetes. *Diabetes care* 31(2): 3194-3201.

Shulman, NB., Ford, CE., Hall, D *et al* (1989). Prognostic value of serum creatinine and effect of treatment of treatment of hypertension on renal function: results from the hypertension detection and follow-up program. *Hypertension* 13 (5 suppl):180-193.

Silver, J (2000). Molecular mechanisms of secondary hyperparathyroidism. *Nephrol Dial Transplant* 15 (suppl 5): 52-57.

Slatopolsky, E (1998). The role of calcium, phosphorus and vitamin D metabolism in the development of secondary hyperparathyroidism. Nephrol Dial Transplant 3: 3-8.

Slatopolsky, E., Finch, J., Denda, M., *et al* (1996). Phosphate restriction prevents parathyroid cell growth in uremic rats. High phosphate directly stimulates PTH secretion invitro. *J clin invest* 97: 2537-2540.

Smith, S., Julius, S., Jamerson, K., *et al* (1994). Haematocrit levels and physiologic risk factors in relation to cardiovascular risk factors in Techumseh, Michigan. *J Hypertens* 12: 455-462.

Sokolow, D., Perloff, D (1961). The prognosis of essential hypertension treated consecutively. *Circulation* 23: 697-713.

St. John, A., Dick, I., Rotallack, R., Welborn, T., Prince, R (1994). Relationship between calcitrophic hormones and blood pressure in the elderly. *Eur J endocrinol* 130: 446-450.

Stuvelling, EM., Bakker, SJ., Hillege, HL., Burgerhof, JG., de-Jong, PE., Guns, RO., de-Zeuw, D (2004). C-reactive protein modifies the relationship between blood pressure and microalbuminuria. *Hypertension* 43: 791-796.

Toure, LA., Salissou, O., Chapko, MK (1992). Hospitalization in Niger (West Africa) for complications from arterial hypertension. *Am J Hypertens* 5: 322- 324.

Trevisan, R., Viberti, G (2000). Pathophysiology of diabetic nephropathy: A fundamental and clinical text. 2nd ed. Lippincott Williams and Wilkins publishers, Philadelphia. Pp 898-909.

Tuttle, KR., Puhlman, ME., Cooney SK., Short, K (1999). Urinary albumin and insulin as predictors of coronary artery disease. *Am J Kidney Dis*. 34: 918-925.

US Renal Data System. USRDS (2008). Annual Data Report: Atlas of chronic kidney diseases and end stage renal disease in the US, National institute of health, National institute of diabetes and kidney diseases.

Vasilios, K., Stella, S., Sofia, P., Zoe, R., Parati, G (2010). Mechanism of obesity induced hypertension. *Hypertension Reseach* 33: 386-393.

Wall, U., Jern, C., Bergbrant, A., Jern, S (1995). Enhaced levels of tissue-type plasminogen activator in borderline hypertension. *Hypertension* 26: 790-800.

Wannamethee, G., Perry, IJ., Shaper, AG (1994). Haematocrit, hypertension and risk of stroke. *J intern med*. 235: 163-168.

White, PC (2001). Steroid 11 beta-hydroxylase deficiency and related disorders. *Endocrinol metab Clin North Am*. 30: 61-79.

Willem, HB (1990). Practical management of hypertension. 3rd ed. Kluwer academic publishers, London. Pp 242-249.

Wolf-maier, K., Cooper, RS.,Banegas, JR., Giampuoli, S., Hense, H., Joffres, M., Tuomilehto, J., Vanzzo, D.,Vanecio, F., Kastarinen, M., Primatesta, P., Rodrigues, AF., Stegmyr, B., Thaum, M (2006). Hypertension and Blood pressure levels in six European countries. *JAMA* 289:2363-2369.

Yarnell, JWG., Baker, IA., Sweetnam, PM., Bainton, D., O'Brien, JR., Whitehead, PJ., Elwood, PC (1991). Fibrinogen, viscosity and white blood cell count are major risk factors for ischemic heart disease. *Circulation* 83: 836-844.

Young, EW., McCarron, DH., Morris, CD (1990). Calcium regulating hormones in essential hypertension: importance of gender. *Am J Hypertens* 3: 161S-166S.