SCHOOL OF BIOMEDICAL AND ALLIED HEALTH SCIENCES

COLLEGE OF HEALTH SCIENCES

UNIVERSITY OF GHANA

SERUM MAGNESIUM LEVEL AND RELATED BIOCHEMICAL PARAMETERS IN CARDIOVASCULAR DISEASES AT THE KORLE-BU TEACHING HOSPITAL

BY

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THIS DISSERTATION IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MASTER OF SCIENCE IN MEDICAL LABORATORY SCIENCES DEGREE.

JANUARY, 2018

DECLARATION

I declare that this thesis is my original work and has not been presented for any other awards at any other university. References to other people's work have been duly acknowledged. This work was carried out in School of Biomedical and Allied Health Sciences, University of Ghana.

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ABSTRACT

Background: Serum magnesium (Mg) and other biochemical abnormalities in cardiovascular diseases (CVD) is a global concern as it has been implicated in CVD related morbidity and mortality. However, results from studies of these biochemical abnormalities especially of serum magnesium in CVDs are conflicting and unclear with no data on CVD patients at the Korle-Bu Teaching Hospital; hence the focus of the current study.

Aim: To determine serum magnesium and related biochemical parameters in cardiovascular disease patients at the Korle-Bu Teaching Hospital.

Method andMaterials: This research was a case-control study design with a convenience and systematic random sampling technique at the Department of Medicine and Therapeutics of the KBTH. A total number of 128 participants including sixty (60) cases with various cardiovascular diseases and 68 control subjects were recruited after their informed consent and ethical clearance (SBAHS/10504897/AA/MLS/2015-2016) was obtained. Four (4) ml fasted blood samples were taken for biochemical analysis. Serum Mg, Ca, K, PO_4, CK, HDL, LDH, and FBG were analyzed in the laboratory using dry Chemistry auto Analyzer (Vitrios 5.1 FS by OrthoClinicals-USA). The data of this study was recorded as protected health information (PHI) and analysed with version 20 of Statistical Package for the Social Sciences (SPSS).

Results: The average serum magnesium level was lower significantly in the CVD patients (0.82±0.16) as compared to the control (0.87±0.07; p-value = 0.02); and magnesium abnormalities (both low and high) were positively associated with CVD cases resulting in an odds ratio (OR) of 5.42 (CI = 1.45 – 20.26; p-value = 0.01). Even though the trend of higher odds of outcome persisted when the exposed group was treated as those having only high magnesium levels, the odds ratio was not statistically significant (1.74 (0.19 – 21.38; p-value = 0.54). With an adjusted OR from binary logistics of systolic blood pressure (SBP) increment of

ten (10) and a body mass index (BMI) increment of five (5), magnesium categories (in terms of low, normal and high values) were found to be associated with inorganic phosphate categories, adjusted calcium, creatinine kinase (CK) categories, diastolic (DBP) categories, albumin (ALB) categories and total cholesterol (Tchol) categories. By this the study established that there was a positive association between serum magnesium level and other biochemical parameters measured ($p = 0.01$). The study parameters, thus, had strong evidence against the null hypothesis. **Conclusion:** The cardiovascular disease patients and controls in this study were adult with majority having secondary school education and of Christian religious background. The study established statistically that there was a significant positive association between mean serum magnesium levels in the controls and cases. Despite this significance, further statistical analysis in accounting for confounding variables with or without rules established that there was a significant relationship between serum magnesium levels in the studied cases and controls. The study established that serum magnesium level was also positively associated with other biochemical parameters measured. The study also established a 13.33% increase risk of CVD prevalence in the participants with low magnesium levels. The current study has further provided information on the relationship between the serum magnesium levels and other biochemical markers in the investigations in CVD subjects and how adjusted odds ratio with SBP increments of ten (10) and BMI increment of five (5) could make magnesium levels closely relate with potassium, phosphate, calcium, CK, DBP, albumin and total cholesterol.

DEDICATION

To **God A**lmighty whose abundant grace has seen me through with this work, **be the glory, great things he has done**. This work is dedicated to my wife, Mercy Bonakor, for the tremendous sacrifices made and children; Semefa, Senyo, Seyram and Selikem

ACKNOWLEDGEMENT

I thank God Almighty for taking me through this programme successfully and my wife, Mercy for the tremendous sacrifice smade and children; Semefa, Senyo, Seyram and Selikem for the patience and support throughout this study.

I express my deepest gratitude goes to Dr. Seth Dortey Amanquah of the School of Biomedical and Allied Health Sciences, for his able supervision, encouragement, patience and guidance in the course of the study.

I further wish to express my sincerest gratitude to Dr. Albert Akpalu of the Department of Medicine and Therapeutics and School of Medicine and Dentistry for his co-supervision, insightful contribution, support and encouragement.

I am all time grateful to Dr. Grace Korkor Ababio of Biochemistry Department, Rev. Dr. Charles Antwi-Bosiako of Physiology Department, and Mr. Confidence Blinksen for their statistical analysis, constructive critiquing and contribution in making this work a reality. I also wish to acknowledge Prof. Nafiu Amidu and Rev. Dr. Thomas Ndanu for their guidance, counsel and tutelage in the result analysis and discussion.

Finally I wish to thank Hospital Management for the opportunity, staffs of Central Laboratory Service, various Units of the Department of Medicine and Therapeutics, SME, volunteered subjects as well as friends and families who in diverse ways helped me produce this work.

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LIST OF ABBREVIATIONS

AHA……America Heart Association

ASA……..…….America Stroke Association

Ca……Calcium

Ca^{2+}…….Calcium Ion

CCF……...…….……Congestive Cardiac Failure

CHD……Coronary Heart Disease

CK…….……..…Creatinine Kinase

CVD……Cardio vascular Disease

CVH……..……..Cardiovascular Health

FBG…….Fasting Blood Glucose

HDL……High Density Lipoprotein

HHD…….……Hypertensive Heart Disease

HIV…….…….Humane Immune Virus

IFG……...…….Impaired Fasting Glucose

IGT……..…..Impaired Glucose Tolerance

IHD……...Ischaemic Heart Disease

K^{+}……..Potassium Ion

K……Potassium

KBTH.....Korle Bu Teaching Hospital

LDL……..Low Density Lipoprotein

Mg……Magnesium

Mg^{2+}.....Magnesium Ion

Na^{+}……Sodium Ion

Na……Sodium

NCD……...Non-Communicable Disease

PO_4.....Phosphate

TG……..……Triglyceride

WHF……..World Heart Foundation

WHO……World Health Organisation

WLE……..World Life Expectancy

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Cardiovascular disease (CVD) refers to diseased conditions that entail the blood vessels including veins, arteries and capillaries or the heart, or both. In other words these are diseases that affect the cardiovascular system (World Health Organization, 2011). Cardiovascular disease includes among others congestive cardiac failure (CCF), hypertensive heart disease (HHD), ischemic heart disease (IHD) and stroke (World Health Organization, 2011).Cardiovascular disease as a major non-communicable disease (NCD) worldwide has been on the increase over time and this has become a burden. Cardiovascular disease is responsible for nearly 10% of all deaths globally at the commencement of the 20th century (Agyemang *et al*, 2012) and the percentage rose to approximately 30% by the start of the 21st century (Mbewu & Jean-Claude, 2006**).**

For the past 6 decades among the high-income countries, cardiovascular disease was ranked the highest among the leading causes of death. However, it has now become the main cause of death in most countries described as having low and middle income in their gross domestic product (Mbewu & Jean-Claude, 2006). It is projected that about 80% of all deaths occurring globally as a result of cardiovascular diseases are taking place in such developing countries. The rise in deaths due to CVD, accompanied by the continual high death rate emanating from contagious diseases in these countries is referred to as the double disease burden by the experts (Mbewu & Jean-Claude, 2006; de-Graft, 2007). Cardiovascular disease is therefore one of the topmost two attributes of death following diarrheal diseases in Ghana (WHO, 2010).

Cardiovascular disease was reported as the primary cause of death in 1991 and 2001 in Ghana (Accra) and it has since remain a leading cause of mortality in the country (Agyei-Mensah & de-Graft Aikins, 2010; Agyemang, 2015). It is believed among a cross-section of people (indigenes) and some experts in Ghana that CVD is not common and therefore, did not pose any grievous public health difficulties (de-Graft Aikins, 2007). Furthermore, Ghana's healthcare system is not equipped with the needed means to tackle the dual burden of non-communicable diseases and the commonplace acute communicable diseases (de-Graft Aikins, 2007). Epidemiologic surveillance has been noticed to be crucial in assessing the quantum of ailment in the population so as to prevent this from escalating further (Gordis, 2009). It is established that population-based data is more often appropriate for these types of observations as it reveals the magnitude of CVD in a particular country. However, this information is hard to come in a lot of developing countries and Ghana is no exception. There has been an institution of demographic and surveillance systems in 3 ecological areas in Ghana (Navrongo, Kintampo and Dodowa) to collect such data, however these opportunities provided have not been fully taken advantage of (Oduro *et al*., 2012). Hospital records have however become a way of monitoring CVD mortality in the absence of such data (Gordis, 2009). Notwithstanding the added input, biochemical markers have been implicated in CVD but data seem conflicting. It is expedient to know the characteristics of these biochemical parameters and investigate as such.

The human body contains an abundant and ubiquitous amount of magnesium. It plays very crucial roles in cellular physiology as well as cellular and biochemical processes that regulate cardiovascular function. It also functions by controlling the tone of the vascular smooth muscle, myocardial excitability and functions of endothelial cells. This makes magnesium very essential to the pathogenesis of a lot of cardiovascular systems disorders such as coronary artery disease,

congestive heart failure, atherosclerosis, hypertension and cardiac arrhythmias. Previous studies have indicated that magnesium was very instrumental in the pathogenesis of cardiovascular diseases. Consumption of low magnesium was established to be linked to future possibility of the following diseases; hypertension (Witteman, Grobbee, Derkx, Bouillon, de Bruijn, & Hofman, 1994), stroke (Larsson *et al*., 2012) and diabetes (Meyer *et al*., 2000). Metabolic syndrome was strongly and positively correlated with reduced serum magnesium concentrations (Rotter *et al*., 2015). Low serum magnesium levels were additionally recounted as a predisposing factor for the commencement and progress of type 2 diabetes mellitus (Kao *et al*., 1999) and coronary heart disease (CHD) (Liao *et al*., 1998). Also, low serum magnesium is also linked to increased cardiovascular mortality in different groups (Adamopoulos *et al*., 2009; Sakaguchi *et al*., 2014). An appropriate magnesium administration averts intracellular reduction in the levels of magnesium, potassium and high energy phosphates thereby improving metabolism in the myocardium; preventing intramitochondrial calcium influx or buildup and decreasing susceptibility to free radicals derived from oxygen. Magnesium, therefore, can influence the tone of the vascular system, anti-aggregation of platelet and the cascading coagulation systems,the severity of cardiac arrhythmias, the size of infarct scar, endothelial function, lipid metabolism, myocardial infarction and cardiovascular failure (Shechter & Shechter, 2013).

The other protective effects of magnesium in CVD include coronary and systemic vasodilation, enhanced angiogenesis, improved exercise duration and cardiac performance, inhibition of reperfusion injury, catecholamine inhibition, and mild reduction of blood pressure and reduced systemic vascular resistance (Shechter & Shechter, 2013). However, a study by Khan *et al*. (2013) concluded their proposition of low serum magnesium concentration being a veritable predisposition factor for one to develop high blood pressure or cardiovascular disease (Khan,

Lubitz, Sullivan, Sun, Levy, & Vasan, 2013).Low serum potassium is established to be a strong and objective predictor of death in congestive cardiac failure (Salah *et al.*, 2015). The concentrations of magnesium and potassium in both plasma and muscle are decreased in congestive cardiac failure (Macdonald & Struthers, 2004). Therefore, a patient that responded to treatment showed a significant rise in concentration of intracellular potassium (Macdonald & Struthers, 2004).

According to reports from other studies conducted it was indicated that serum calcium may interfere with the composition of lipid fractions such as total triacylglycerol, low density lipoprotein (LDL) and level of high density lipoprotein (HDL) in CVDs (Reid *et al.*, 2002). Due to the fact that a disordered lipid is a key predisposition for coronary heart disease (CHD), the association between the levels of serum calcium and CHD needs extensive study. The correlation of serum calcium with total serum cholesterol has also been established by other studies (Gallo *et al.*, 2016) and with levels of one's blood glucose (Saltevo *et al.*, 2011) and as a result obviously linked with the pathogenesis of metabolic syndrome. Some studies reported that middle-aged men with high serum calcium have an independent, prospective predisposition for myocardial infarction (Gallo *et al.*, 2016). It is reported in other studies that people with type 2 diabetes mellitus have an increased chance of developing CVD including IHD (Deedwania & Fonseca, 2005; Mazzone *et al.*, 2008).

It is also reported that clogging of the vascular system by atherosclerotic plaque (atherosclerosis) usually predates the onset of clinical diabetes mellitus (Hu *et al.*, 2002). Also, evidences from experimental studies have proved that there is an association between impaired glucose tolerance (IGT) and increased morbidity and death of cardiovascular diseases (Nathan *et al.*, 2007).

Despite this relationship observed between impaired fasting blood glucose (IFG) and the possible predisposition to having CVD and IHD, there is still no certainty to the association (Liu *et al.*, 2007, Levitzky *et al.*, 2008).

There is a reported significantly high serum Creatine kinase (CK) levels in CVD patients as compared to the controls (Al-Hadi & Fox, 2009). Therefore, it is anticipated that stroke-related myocardial injury is as a result of high CK levels which served as a biological indicator. It has further been indicated that certain people suffering from ischaemic stroke, subarachnoid hemorrhage, and head trauma have an elevated creatine kinase activity in the nonexistence of any clinically evident acute coronary syndrome (Peppes *et al*., 2008; Manea *et al*., 2015).

In populations with high chronic kidney diseases, phosphate (PO_4) levels are consistently known to be linked with cardiac calcification, CVD, and death (Foley *et al.*, 2009). High phosphataemia is a key predisposing factor for cardiovascular situations, calcification of vascular system and cause of death among clients with or without kidney disease (Kendrick *et al.*, 2011). The established processes by which elevation in the level of phosphate result in undesirable outcomes are not yet completely understood as serum phosphate levels within the referenced laboratory range are even correlated with a higher threat of cardiovascular events and death. However, existing scientific evidence suggests that there is a direct action of phosphate on calcification of the vascular systems and modulation by such major hormones as calcitriol and fibroblast growth factor-23 (Kendrick *et al.*, 2011).

In spite of the convincing epidemiologic associations between biochemical parameters like phosphate excess and cardiovascular disease, not much studies has been conducted to establish

this relationship to confirm or otherwise the risks and benefits of treatment. It is anticipated that some biochemical variables like the levels of serum phosphates can cover the traditional reference range and result in condition of CVD. Investigating biochemical markers e.g. PO_4 levels, in the CVDs may be important since many clinical mediations may be appropriate for the primary or secondary avoidance of CVDs.

1.2 Problem Statement

In both the advanced and developing countries, cardiovascular disease is on the increase although the disease burden and mortality may be different (WHO, 2005). A recent study done in Ghana also reported increasing burden and mortality of cardiovascular disease (Sanuade *et al.*, 2013). Studies have suggested some relationship between cardiovascular health and magnesium (Shechter & Shechter, 2013); however very little information on the specific association between cardiovascular disease and serum magnesium levels is currently available in Ghana. Available literature reveals that most of these works were done in advanced countries and some evidence that there is increasing cardiovascular disease burden in Ghana. However, there are no available data on the specific relationship between magnesium and cardiovascular diseases (CVD) in Ghana to the best of my knowledge.

1.3 Justification

Cardiovascular disease (CVD) being a debilitating disease with devastating financial consequences, clients may benefit from early magnesium supplementation to ameliorate the severity of relevant CVD conditions. Data generated may offer policy makers and other health professionals the opportunity to build on the derived baseline knowledge to provide further

solutions. Laboratory request for magnesium is generally low among the requesting physicians in KBTH in spite of the reported benefits that can be available to the affected clients/patients.

1.4 General Aim

To determine serum magnesium levels and related biochemical parameters in cardiovascular disease attending Korle-Bu Teaching Hospital and compare with apparently healthy subjects.

1.5 Specific Objectives

The specific objectives were to:

1. Determine the demographic status of subjects
2. Determine serum magnesium levels in cardiovascular disease.
3. Determine serum levels of biochemical parameters in cardiovascular disease
4. Determine the relationship between serum magnesium levels and other biochemical parameters in the studied subjects.
5. Determine the likely risk of magnesium level in developing cardiovascular disease.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Cardiovascular Disease

Cardiovascular disease (CVD) is defined by the American Heart Association (AHA) as diseases of the heart and its blood vessels as well as the vascular diseases of the brain (Kasner & Sacco, 2013). Cardiovascular diseases are accountable for more than 17.3 million deaths annually and for 31% of all deaths in the world accounting for the number one cause of death (WHO, 2008). This condition is the prominent non-communicable disease with almost 50% of the estimated 36 million deaths due to non-communicable diseases being as a result of CVDs. Approximately 10% of the worldwide burden of disease is ascribed to CVD. An important predisposing comorbidity of heart and respiratory disease, stroke, diabetes and cancer include the tobacco smoking, harmful alcohol use, lack of physical exercise and consumption of unhealthy diet (Mendis *et al*., 2011).

Cardiovascular diseases are also defined as a collection of debilitating disarray of the heart and its associated blood vessels with destructive complications in individuals who have been affected. This has become the primary basis of non-communicable disease worldwide and comprise of: cerebrovascular disease (stroke) which essentially alters the vessels that supply blood to the brain; coronary heart disease which affects the vessels that supply blood to the heart muscles; rheumatic heart disease which comes about as a result of damage to the heart muscle and heart valves from rheumatic fever and peripheral artery disease caused by streptococcal bacterial infection which affects the vessels supplying blood to the arms and legs. It also includes congenital heart disease which is as a result of distortion of structures of the heart present at birth, deep vein thrombosis and pulmonary embolism resulting from blood clots in the veins of

the leg, which can dislodge and translocate to the heart and lungs (WHO, 2007). Heart attacks and strokes are generally acute events and are primarily caused by an obstruction that prevents blood from flowing to the heart or brain. This is as a result of an accumulation of fatty deposits on the internal walls of the blood vessels. Stroke attacks are usually caused by bleeding from a blood vessel in the brain or by blood clots (WHO, 2007).

2.2 Pattern of CVD in Developed and Developing Countries

Ironically, the burden of CVD-related morbidity and mortality has rather been increasing although the sub-Saharan Africa region is considered to consist largely of the youngest human populations internationally. Between 1990 and 2013, Moran *et al.* (2014) reported in their study that CVD-related deaths were the highest in that region as compared to other parts of the world that remained steady or declined. According to Livesay (2007), CVD-related deaths were the most important cause of death among the populations that aged above 45 years and thereby accounted for nearly 9.2% of all deaths occurring in the African region in 2001. It was further reported that about 7-10% of all adult admitted with medical conditions to health facilities in Africa were as a result of CVDs with heart failure alone representing approximately 3-7% (Mocumbi, 2012). It was reported that cerebrovascular accident (CVA) generally known as Stroke death rates are similar to that of the developed countries although Africa has the least coronary artery disease (CAD) death rates (WHF, 2015). In 2010, Stroke was ascribed as the main cause of CVD mortality and death in Africa. In that singular year, stroke accounted for approximately half a million deaths which represented 4.4% of all deaths in Sub-Saharan Africa (Ofori-Asenso & Garcia, 2016). The main acquired CVD in children and adolescents in Africa is rheumatic heart disease. The prevalence of this category of CVD is the highest in the world accounting for about 15-20 per 1,000 population (WHO, 2005). On the whole, more than half of

the CVD-related deaths in Africa happen between the ages of 30-69 years in many people. This is a decade or more lower than comparable group in the advanced world (Agyemang, 2015). It is projected that the burden of CVD will keep rising in Africa and by the year 2020 will become doubled the burden in 1990 (Mbewu & Mbany, 2015). Globalization has partly been attributed for the increasing burden of CVDs and its related diseases or its predisposition on the African continent.

2.3 Burden of Cardiovascular Disease in Sub-Saharan Africa

Cardiovascular disease is the first of the top 10 causes of death in Africa and complications such as stroke and renal disease are becoming progressively more common. Modifiable predisposing factors like obesity, hypertension, tobacco use, physical inactivity and raised cholesterol levels are known key drivers of the disease (Abanilla *et al.*, 2011). Evidence has it that countries within Sub-Saharan African region are presently undergoing a swift epidemiological transitions typified by an increase in altering traditional lifestyle characteristics afforded by globalization and urbanization (BeLue *et al.,* 2009). It was estimated in 2005 that about 17.5 million people perished from CVD. This represented about 30% of all worldwide deaths for which approximately 80% emanated from developing countries with sub-Saharan Africa inclusive (WHO, 2007). The Sub-Saharan Africa consists of regions within Africa that are completely or partly located south of the Sahara Desert with Ghana, Nigeria and South Africa inclusive. These countries are presently experiencing a raised incidence of NCDs, especially CVD due to rapid epidemiological transitions with increased urbanization (Kadiri & Salako, 1997) and changing lifestyle factors (BeLue *et al.,* 2009). South Africa's CVD related death is reported to be the second major cause of death after HIV representing 40% of deaths among adults (Peer *et al.*, 2008).

Several other literature reviews also show that the incidence of cardiovascular disease (CVD) has risen considerably to become the primary cause of morbidity and mortality in the sub-Saharan Africa (Addo *et al.,* 2007; Vendathan & Fuster, 2008 & BeLue *et al.*, 2009). Cardiovascular disease (CVD) as a main element of non-communicable diseases is costly in terms of management and interferes with socioeconomic activities. This will continue to exhaust already scarce health resources in the Sub-Saharan African region. The increase in economic situation has worsened the poverty gap. Also with the perennial health-care worker shortages across the region, the response to this escalating CVD burden has seriously been hindered and this can pose a new public health challenge in the region. (Kadiri, 2005; Seedat, 2007 & BeLue, 2009). For this reason cost-effective ways to detect, control and prevent CVD are immediately required.

2.4 Burden of Cardiovascular Disease in Ghana

In Ghana, CVD remains the principal cause of death within the last two decades (de-Graft Aikins, 2007). The socioeconomic challenge of CVDs is on a steady increase in Ghana for which globalization is undoubtedly reported to be contributing to in no small ways by driving significant behavioural changes across the country (Ofori-Asenso & Garcia, 2016). The WHO has also identified CVDs as the leading cause of death among the non-communicable diseases in Ghana (WHO, 2011) and only second after infectious diseases including diarrheal illnesses (WHO, 2010).

A study conducted within a 5year period between January 2006 and December 2010 at Korle Bu Teaching Hospital (KBTH) of Ghana revealed that cardiovascular deaths diagnosed at autopsy during this period accounted for about 22.5% of all deaths (Sanuade *et al.*, 2014). This figure is

closely collaborated by World Life Expectancy publication of 17.59% (32,922) of all deaths attributable to CVD accounted for by stroke, coronary heart disease (CHD) and hypertension. According to published WHO data in 2014, coronary heart disease deaths in Ghana accounted for about 6.48% of total deaths registered. The age adjusted death rate was 96.73 per 100,000 population and making Ghana ranks number 75 in the world (WLE, 2016).

Non-communicable diseases (NCDs) in Ghana accounted for an estimated 39 per cent of all mortality in 2008 and in the same year, the most prevalent NCDs were cardiovascular diseases accounting for 18% of all diseases reported (Commonwealth Health, 2016). Review of many literature available show that cerebrovascular accidents (stroke) constitute a significant source of CVD morbidity and mortality in Ghana. In a two-year study by Agyemang *et al.* (2012) on the incidence of stroke in the Ashanti region of Ghana, it was reported that stroke alone, of the CVD burden in Ghana constituted 9.1% of total admission of all medical adults and 13.2% of all medical adult deaths within that period. In an older but similar cross-sectional study of fatal stroke cases over a 5-year period by Wiredu & Nyame (2001) in persons aged 20 years and above and confirmed at autopsy in Korle Bu Teaching Hospital, mortality from stroke constituted 11% within the 5-year period of 1994 to 1998. Compared with the last study done in 1981 on the same parameters, stroke still remains an important cause of CVD deaths in Accra, Ghana.

2.5 Cardiovascular Health

The ultimate cardiovascular health is defined by American Heart Association as the presence of both ideal health behaviors and ideal health factors. Ideal health behaviors are characterized by the avoidance of smoking, good physical activity, healthy diet and a body mass index below 25

kg/m^2. Ideal health factors are characterized by fasting plasma glucose concentration below 5.6mmol/L, untreated blood pressure below 120/80 mmHg and (untreated total cholesterol below 5.2mmo/L (Lloyd-Jones *et al*., 2010).

In this study, cardiovascular health (CVH) is defined as the absence of cardiovascular disease or absence of disease of the heart and its blood vessels as defined above. The perfect cardiovascular health is characterized by good health behaviours such as non-smoking habit, good body mass index, adequate physical exercise, healthy diets, and acceptable referenced levels of total cholesterol, fasting blood glucose and normal range of blood pressure. Conversely, these metrics also serve as good CVD risk factors. These ideal cardiovascular health metrics were goals set by American Heart Association (AHA) to enhance the cardiovascular health of Americans by 20% by 2020 (Folsom *et al*., 2011).

2.6 Aetiology of Cardiovascular Disease

Recent advances in experimental and epidemiological research have proven the crucial role magnesium ions (Mg^{2+}) play in the origin and cause of cardiovascular pathologies. Hypomagnesemia in human subjects is frequently linked to an imbalance of electrolytes such as sodium (Na^{+}), potassium (K^{+}) and calcium (Ca^{2+}). Different kinds of heart diseases, for example, ischemic heart disease, sudden cardiac death, atheroscelerosis, congestive heart failure, cardiac arrhythmias and ventricular complications as seen in diabetes mellitus come about as a result of unusual dietary insufficiency of Mg^{2+} coupled with defects in the metabolism of $Mg2^{+}$. Magnesium deficiency leads to advanced vasoconstriction of the coronary vessels resulting in a drop in oxygen and nutrient transfer to the cardiac myocytes (Chakraboti *et al*., 2002)

Balance in body electrolytes has been considered a key factor necessary to maintain cardiovascular stability especially congestive heart failure (CHF). Amongst the shared electrolytes, the importance of magnesium has been a subject of debate due to problems associated with accurate and precise measurement and other related factors such as aberrations in other electrolytes. The serum magnesium concentration characterizes less than 1% of total body stores and is not reflective of total-body magnesium concentration. Magnesium is a significant cofactor in numerous enzymatic activities in the body and contributes to steady cardiovascular haemodynamics and electrophysiologic functioning. Its deficiency is common and is usually linked to risk factors and heart failure complications (Douban *et al*., 1996).

Epidemiological studies conducted in several nations including the USA, Finland, South Africa, Canada, England, France, Germany and Netherlands have shown, for example, that drinking water with low magnesium content is linked to the incidence and death from coronary artery disease (Seelig & Rosanoff, 2003; Shechter & Shechter, 2013).The highest risk for coronary artery disease (CAD) has been associated with subjects with low serum magnesium concentration (Liao *et al*., 1998). The National Health and Nutrition Examination Survey (NHANES) epidemiologic study established an inverse association of serum magnesium and death from CAD and all-cause mortality (Ford, 1999). Eating food that has low magnesium content was found to escalate the incidence of CAD by 2.1% in comparison to high magnesium content (Abbott *et al.,* 2003). Low serum magnesium levels were linked to a threefold incidence of cerebrovascular accident (CVA) in peripheral artery disease patients compared to those with high levels of magnesium concentrations (Amighi *et al*., 2004).

An inverse association exists between food magnesium content and the incidence of metabolic syndrome (He *et al*., 2006). Prospective epidemiologic studies have described various relationships between magnesium and the risk of CVD (Qu *et al*., 2013; Abbott *et al*., 2003). Commonly, a stronger correlation existed for serum rather than dietary magnesium. The relationship between serum magnesium and risk of CAD seem to be stronger for fatal events rather than non-fatal ones, which can possibly be explained if magnesium has a protecting role against fatal ventricular arrhythmias and thus sudden cardiac death (SCD) (Ford, 1999). This theory was further buttressed by ecologic studies, which reported an inverse relationship between regional drinking water hardness and sudden cardiac death (Chiuve *et al*., 2011) and autopsy and many prospective studies have shown that lower myocardial magnesium levels were found in SCD victims as compared with death from trauma (Abbott *et al*., 2003). However, researchers from the Netherlands found no evidence for an overall significant association between tap water hardness, magnesium or calcium concentrations, and IHD mortality or stroke mortality (Leurs *et al.*, 2010).

The magnesium content of foods in the USA has dropped over the last 20 years and the incidence of CAD is also on the rise (Shechter & Shechter, 2013).It has been put forward that the effect of magnesium in preventing CVD may be attributed to a decline in inflammatory response. Arise in extracellular magnesium levels leads to phagocyte and endothelial cell activation (Shechter & Shechter, 2013). Inflammation caused by experimental magnesium deficiency is the mechanism that induces high plasma triglyceride levels and proatherogenic changes in lipoprotein profile. In magnesium deficiency states, endothelial cells are known to actively lead to inflammation. There is an existing inverse relationship between intake of magnesium and markers of systemic inflammation and endothelial dysfunction in healthy persons (Song *et al*.,

2007). Animal models of magnesium deficiency have been proven to induce a clinical manifestation of inflammatory syndrome characterized by leukocyte and macrophage activation, inflammatory cytokines release and acute phase proteins as well as extreme free radicals production (Pachikian *et al*., 2010; Lin *et al*., 2010). Several mechanisms may act cumulatively and or synergistically to protect myocytes and that establishes the basis for supplementation with magnesium in patients with heart disease (Shechter & Shechter, 2013). This is in agreement with many other studies (Seelig & Rosanoff, 2003; Shechter & Shechter, 2005; Agarwal, 2016).

2.7 Types of Cardiovascular Diseases (CVDs)

2.7.1 Ischaemic Heart Disease

Ischaemic heart disease (IHD) has various alternative names. They include coronary artery disease (CAD) or coronary heart disease (CHD). It is a condition that affects the supply of blood to the heart. It is a group of heart diseases that include myocardial infarction (MI),stable angina, unstable angina and sudden cardiac death (SCD) (Wong, 2010). Within the cardiovascular diseases, IHD is the most common kind (Moran *et al.*, 2014). The coronary arteries supply oxygenated blood to the heart muscles and no alternative blood supply exists. Therefore, a thinning or blockage in the coronary arteries known as atherosclerosis reduces the supply of blood to the heart and its muscles resulting in ischemia of the muscles causing angina pectoris. The risk factors of the condition include high blood pressure, lack of exercise, smoking, obesity, hyperlipaedemia, diabetes, excessive alcohol intake, and poor diet among others (Mendis *et al*., 2011 and Mehta *et al.*, 2014).

As a result of limitation of oxygenated blood flow to the heart and its muscles due to atherosclerotic plague formed from calcium phosphate deposition with cholesterol in the arteries,

the condition of ischaemia results. This is myocardial cell starvation secondary to oxygen deprivation resulting myocardial infarction (MI) commonly known as heart attack. This leads to damage, scarring and death of the heart muscles. Myocardial damage and scarring may cause failure of heart muscle regrowth. Chronic high grade stenosis of the coronary arteries may induce transient ischaemia which may also lead to the initiation of a ventricular arrhythmia. The resultant ventricular arrhythmia may end in ventricular fibrillation leading to death (Ambrose & Singh, 2015).

2.7.2 Hypertensive Heart Disease (HHD)

Hypertensive heart disease (HHD) refers to complications of heart conditions caused by high blood pressure (Badii, 2017). While there are a number of definitions of hypertensive heart disease in the medical literature, according to Alegría-Ezquerra *et al*, 2006), the term is very commonly utilised in the classification of diseases. In 2013 hypertensive heart disease resulted in 1.07 million deaths as compared with 630,000 deaths in 1990 (GBD, 2013). According to the medical dictionary, MedicineNet (2016), Heart failure (HF) as a major outcome of hypertensive heart disease is often referred to as congestive heart failure (CHF) and this is as a result of theheart’s inability to pump blood proficiently and adequately to sustain flow to meet the body's requirements. Heart failure is as a result of damaging or over loading of the heart muscle thereby minimizing its effectiveness. Conditions that can cause this disease include myocardial infarction (in which the heart muscle does not get sufficient oxygen and dies), hypertension (which increases the force of contraction needed to pump blood) among others.

Table 0:1**Stages of elevated Blood Pressure and Hypertension**

Category	Systolic BP (mmHg)	Diastolic BP (mmHg)
Optimal	**< 120**	**< 80**
Prehypertension	**120-139**	**80-89**
Stage I	**140-159**	**90-99**
Stage II	**>160**	**>100**

Source: Chobanian *et al*., 2003

2.7.3 Stroke/Cerebrovascular Accident (CVA)

A stroke sometimes called a cerebrovascular accident (CVA) is one of the most common medical emergencies related to the heart. This occurs when blood is deprived from reaching an important area like the brain resulting in the death of the brain cells due to lack of oxygen and glucose needed to survive. MedicineNet (2016) defines Cerebrovascular accident as the sudden death of some brain cells as a result of the absence or insufficient oxygen flow when the flow of blood to the brain is impaired by blockage or rupture of an artery to the brain.

However, the American Heart Association (AHA) and American Stroke Association (ASA) updated definition of stroke for the 21st century defined Stroke as an episode of neurological dysfunction caused by focal cerebral, spinal or retinal infarction. It further described central nervous system infarction as brain, spinal cord or retinal cell death due to ischaemia based on either pathological, imaging or other objective evidence of cerebral, spinal cord or retinal focal ischaemic injury in a defined vascular distribution; or clinical evidence of cerebral, spinal cord or retinal focal ischaemic injury based on symptoms persisting for at least 24-hours or until death, and other aetiologies excluded (Kasner & Sacco, 2013).

There are two types of stroke; ischaemic and haemorrhagic strokes. The ischaemic strokes is as a result of clots accumulating in the blood vessels of the brain, in blood vessels leading to the brain (thrombotic strokes), or blood clots or other substances such as bone marrow fats released by means ofa fracture, bacterial clumps as in endocarditis and air particle in blood vessels elsewhere in the body and then get transported to the brain (embolic stroke). These clots from any of the various sources can block the blood flow to the cells of the brain thus causing ischaemic damage and death to the brain cells via oxygen and glucose deprivation. Haemorrhagic strokes commonly due to high blood pressure and brain aneurysm occur when there is a burst of blood vessel in the brain leading to blood draining into the brain tissue and causing destruction to the cells of the brain (Gund *et al*., 2006).

2.8 Serum Magnesium Levels in CVD

Being an active mineral biologically, magnesium is commonly found in such foods as unpolished grains, green leafy vegetables and nuts. In most western countries the consumption of magnesium is reported to be inadequate as a lot their food eaten is processed. (Ford & Mokdad, 2003; Song & Liu, 2012). Low dietary intake, high consumption of processed foods and certain diseased conditions usually account for hypomagnesemia (Swaminathan, 2003). Hypertension and other cardiovascular diseases as well as nerve disorders have been treated using magnesium for years (Gums, 2004; Houston & Harper, 2008).The levels of magnesium in the body are closely regulated by the parathyroid hormone. Excess magnesium levels are gotten rid of through faecal excretion and urinary system (Smith, 2009). Majority of the stock of magnesium in the body are bound and unavailable while only close to 1% are available in the blood since serum levels are tightly regulated through uptake, then renal and fecal excretion. To this end, about 310

to 350 mg of magnesium has been recommended for daily consumption. Elemental magnesium supplementation for adult male and female has been capped at 350 mg per day. As a supplement, magnesium is generally reported to be a safe. There are virtually no recorded incidences of excessive dietary magnesium to be the cause of any adverse effect. However, wanton consumption of supplemental magnesium may lead to stomach cramps and incidence of diarrhea (NIH, 2005). In the same report, the National Institutes of Health reported that the signs of excess magnesium more than five times the daily recommended dose may cause signs and symptoms as those of magnesium deficiency. These signs and symptoms may include mental confusion and instability, nauseating feelings, losing appetite, having diarrhea, breathing difficulties, muscle weakness (NIH, 2005).

In an ARIC Study in four US communities involving 15,248 subjects, men and women aged between 45 and 64 of both blacks and whites, Ma *et al.,* (1995) concluded that hypomagnesaemia and low dietary intake of magnesium may have contributed to the etiologies of CVD including development of atherosclerosis, hypertension and diabetes. In their study, both serum and dietary magnesium levels were lower in blacks than whites. The average serum magnesium levels were significantly lower in the cases with CVDs compared to the controls that had no any CVD conditions.

2.9 Protective Roles of Magnesium in CVD

Exogenous magnesium administration helps maintain intracellular magnesium, potassium and high energy phosphates levels. This helps to improve the metabolism of the myocardium; and prevent the influx of calcium intramitochondrially. It also avoids the unintended accumulation of oxygen-derived free radicals as well as reduces their cellular vulnerability. Magnesium in

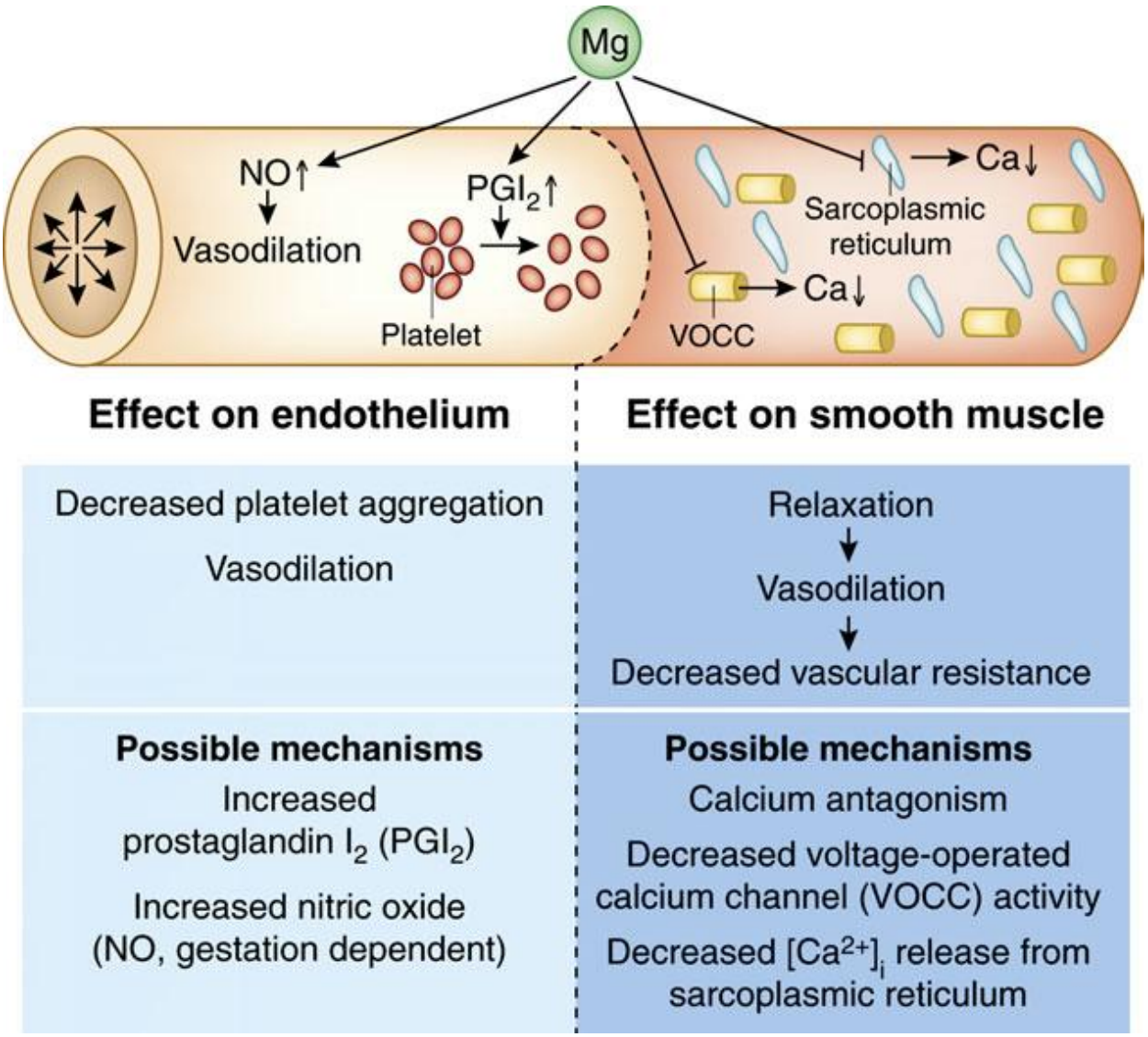
summary, therefore, have a positive effect on the tone of the vasculature, prevent aggregation of platelet, improves the functioning of the coagulation system and endothelium, limit the size of infarct scar, enhance lipid metabolism, prevents cardiac arrhythmias and myocardial infarction as well as reduces cardiovascular failure (Shechter & Shechter, 2013). The other protective effects of magnesium in CVD include coronary and systemic vasodilation, enhanced angiogenesis, improved exercise duration and cardiac performance. Also includes inhibition of reperfusion injury, catecholamine inhibition, mild reduction of blood pressure and reduced systemic vascular resistance (Shechter & Shechter, 2013). In contrast, a study by Khan *et al*., (2010) indicated that their data derived from a huge, community-based cohort failed to agree with the assumption of serum hypomagnesium being a predisposition variable in the development of cardiovascular disease including hypertension.

2.10 Magnesium and Vascular Smooth Muscle Cells (VSMCs)

Hardening of the vasculature as a result of excessive calcium deposition is commonly present in such conditions as atherosclerosis, aneurysms, diabetes, hypertension, and chronic kidney disease (CKD). This observation is found related to elevated predisposition to cardiovascular morbidity and mortality. (Kaye *et al*., 2007). Hypercalcaemia add to the elevated hardness of the vasculature, contributes to its reduced elasticity and decreased distensibility and this is a characteristic feature of the vascular phenotype in hypertension (Dao *et al*., 2005; Kanemaru *et al*., 2008). Hardening of the vasculature as a result of excessive calcium deposition is a firmly regulated course similar to processes involved in the formation of bone (Peacock, 2010). Factors enhancing excessive deposition of calcium in the vascular system include disorders in the metabolism of relevant minerals, particularly in the conditions of hyperphosphatemia and high serum calcium level (Peacock, 2010).

In hypomagnesaemia, vascular calcification may be exacerbated, (Montezano *et al*., 2010) and studies have indicated a direct relationship between hyperphosphatemia, hypercalcaemia, and calcification of the arteries. Results obtained from in vitro observations support this reported relationship. It can be explained that when vascular smooth muscle cells are exposed to high concentrations of phosphate and calcium a dose-dependent elevation in mineralization was noted. This indication is associated with vascular smooth muscle cells differentiation to an osteoblastic phenotype (Speer *et al*., 2009). This process is facilitated by upregulating transcription factors like core-binding factor 1/Runx2, MSX-2 and bone morphogenetic protein-2. These factors are involved in the regular process of bone development. They therefore, control the expression of osteogenic proteins. These osteogenic proteins include osteonectin, alkaline phosphatase, osteocalcin, bone sialoprotein and collagen-1 (Freedman *et al*., 2009). Magnesium inhibits vascular calcification by reducing the expression of osteocalcin and bone morphogenetic protein-2 and related activities. This leads to elevated production of calcification inhibitors such as osteopontin and matrix Gla protein (Montezano *et al*., 2010). These bone-forming transcription factors and proteins can be produced by vascular smooth muscle cells in a culture medium. The effect of vascular calcification was seen increased with high concentrations of calcium, phosphorous, glucose, cytokines, oxidized lipids in the presence of a low concentration of magnesium (Speer *et al*., 2009).Conversely, an alternative process involved in creating a condition of vascular mineralization is through the absence of inhibitors to vascular calcification. They include matrix Gla protein, fetuin-A, osteopontin and pyrophosphate (Scatena *et al*., 2007; Zarjou *et al*., 2009).

Figure 0: IVascular effects of magnesium sulphate



Source: (Euser and Cipolla, 2009).

Magnesium is an important vasodilator of arteries. Magnesium in the vascular smooth muscle usuallycompetes with calcium for the available binding sites. Decreased calcium channel activity is associated with reduced mobilization of intracellular calcium. This results in the relaxation and vasodilatation of the vasculature. Magnesium further promotes high expression of prostaglandin I_2 in the endothelium. This action tend storeduce the aggregation of platelet. Magnesium also

promotes the elevation of nitric oxide production resulting in vasodilatation of the vascular system (Euser & Cipolla, 2009).

2.11 Magnesium and the Vascular Endothelium

The functional patency of the wall of the vasculature is known for the maintenance of the cells of the endothelium. The integrity of the vascular wall is critically important to ensure that vessel permeability is properly controlled and non-agglutination of blood–tissue interface is maintained. Vascular wall patency is also needed for modulation of blood flow and its vascular resistance. Functional integrity of the vascular endothelium further assures proper regulation of the immune and inflammatory reactions (Cines *et al*., 1998). Endothelial dysfunction has been established to play a major role in a variety of diseases including: diabetes and thrombosis (Cines *et al.*, 1998); atherosclerosis and hypertension (Lifton *et al*., 2001). Research supports the hypothesis of endothelial dysfunction as the common connection between risk factors and burden from atherosclerosis (Ross, 1999). The dysfunction of the endothelium has been found to play a role in the following: increasing chemokine secretion and cell permeability to lipids; the process of lesion formation by promoting leukocyte adherence; improving LDL oxidation, migration and platelet activation and stimulating vascular smooth muscle cell proliferation (Ross, 1999). Also, cells of the endothelium play a role in angiogenesis (the branching and sprouting of capillaries from pre-existing blood vessels), which is a firmly controlled event critical in development, reproduction and healing of wound (Folkman, 1995). Endothelial cells are freely exposed to numerous signals (metabolites, cytokines, ions, free radicals, shear stress) due to their strategic position at the boundary between blood and vessels, some of which may stimulate maladaptative changes in function.

Experimental evidence shows that low magnesium status is vital to the pathogenesis of cardiovascular diseases (Seeling & Rasandof, 2003). Hypomagnesaemia which is common in western countries is a common denominator in coronary artery disease (Liao *et al.*, 1998), hypertension (Peacock *et al.*, 1999), thrombosis (Mussoni *et al*., 2001) and diabetes (Barbagallo *et al*., 2007). Oral magnesium therapy to patients suffering from coronary artery disease has been linked to improvement of their endothelial function (Shechter et al., 2000) accompanied by a drop in plasma levels of very low density lipoprotein (VLDL), triglycerides, and apo-B (Geiger & Wanner, 2012). Hyperlipaemia, early atherosclerotic lesions and inflammation have been seen with experimental magnesium deficiency (Rayssiguier *et al.*, 2001). Fortification of drinking water with magnesium led to the inhibition of atherogenesis in APO-E-deficient mice fed on a high cholesterol diet (Ravn *et al.*, 2001). A deficiency in magnesium has been proven to lessen tumour growth through mechanisms that are not clear (Wolf & Cittadini, 1999). Also magnesium facilitates wound healing as high levels of magnesium has been identified in early wound fluid, once movement of cells into the wound is started (Grzesiak & Pierschbacher, 1995). Research based evidence suggests that extracellular magnesium concentrations play an important role in controlling activities of the endothelium (Zhang *et al*., 1993). Deficiency in magnesium also promotes free radical-induced cytotoxicity in the cells of the endothelium (Montezano *et al*., 2010). Again many biological processes at the cellular and molecular levels have been shown to be impacted by magnesium deficiency. The mechanisms are systemic reactions such as inflammation and endothelial dysfunction, as well as changes at the cellular level, such as mitochondrial dysfunction and excessive fatty acid production (Zheltova *et al.*, 2016).

2.12 Magnesium and the Myocardium

Intravenously administered magnesium has been used in the treatment and prevention of several acute cardiovascular diseases (Fawcett *et al.*, 1999). Magnesium functions as a cardiovascular drug together with calcium but possess antagonistic and antiadrenergic properties (James, 1992). The cardiovascular properties of magnesium include its antiarrhythmic effect, direct and indirect dilatation of blood vessels, and possibly myocardial depression. Although there is a direct correlation between plasma magnesium concentration and cardiovascular effects at near steady state, the association between the time courses of magnesium concentration and its effect is poorly understood (Nakaigawa *et al*., 1997). Higher knowledge of this association may account for the design of acute intravenous dose regimens of magnesium for the management of cardiovascular symptoms. It is however uncertain whether the cardiovascular effects of magnesium are facilitated by its intracellular or extracellular concentration or a combination of both (Qu *et al.*, 2013). Magnesium also has effect on some cardiovascular parameters such as myocardial blood flow and myocardial contractility.

Deficiency in magnesium has been linked to the following: enhanced platelet aggregation; induction of severe vascular damage in the heart; increase in blood pressure; enhanced development of atherosclerosis; and vasoconstriction of the coronary arteries (Geiger & Wanner, 2012). A reduced magnesium concentration appears to be complicated in the pathogenesis of ischaemic heart disease by changing lipoprotein composition, thus making individuals prone to atherosclerosis (Montezano *et al.*, 2010). It was reported that, a reduction in infarct size occurred when magnesium was administered to animal models with myocardial infarction (Antman, 1996). According to an autopsy studies done on patients who had died from ischaemic heart disease, it was revealed that there were lower magnesium levels in their myocardium and muscle

in comparison to patients who had died from non-cardiac causes (Gund *et al.,* 2006). Overall intracellular magnesium declines while free ionized intracellular magnesium increases during myocardial ischaemia (Manea *et al*., 2015). In addition, ischaemia leads to intracellular calcium overload and it is hypothesised that administration of magnesium reduces calcium overload for the reason that there was evidence that these two elements are in competition with each another for the same binding sites. Magnesium may be seen as a natural 'calcium antagonist' (Mentezano *et al*., 2010) and can attenuate phosphate-induced apoptosis in vascular smooth muscle cells (Kircelli *et al*., 2012).

2.13 Risk Factors of Cardiovascular Disease

According to WHO (2007), one of the greatest challenges that faces health systems worldwide is the increasing burden of chronic diseases. Cardiovascular diseases are one the common non-communicable chronic disease conditions increasingly affecting the human population. A chronic condition according to WHO is defined as needing and ongoing management over a period of years or decades (Nolte & Mckee, 2008). Cardiovascular risk factors are elements that predispose or increase the chances of developing cardiovascular disease. These factors are generally grouped into two categories, namely, modifiable and non-modifiable risk factors.

With the modifiable risk factors, the individual is able to control the elements; these are preventable risk factors afforded by appropriate lifestyles. The non-modifiable risk factors are genetic or inherited elements outside the control of the individual and are therefore, non-preventable in the development of cardiovascular diseases (WHF, 2016)

2.14 Body Mass Index (BMI)

The body mass index *(*BMI*)* or Quetelet index is an assessment calculated from the mass(weight) and height of an individual. It is defined as the weight of an individual divided by the square of the height of the person, and this is generally expressed in units of kg/m^2. The National Institutes of Health (NIH) continue to utilise BMI as a standard tool for measuring normal weight, overweight and obesity. These classifications serve as an acceptable clinical tool for assessing whether people who are inactive in their daily routines are underweight, overweight or obese with a variety of exceptions including the infirm, elderly, children, and athletes. It is primarily utilised as a way of correlating comparative groups related by their general weight. It remains an important means of estimating adiposity although not very accurate in its measurements. The tool of BMI has also remained in use by WHO as an acceptable standard for measuring obesity statistics for nearly four decades now.

Table 0:2 WHO Obesity Classification

Category	**BMI (kg/m^2)**	
	from	**To**
Very severely underweight		15.0
Severely underweight	15	16
Underweight	16	18.5
Normal (healthy weight)	18.5	25
Overweight	25	30
Obese Class I (Moderately obese)	30	35
Obese Class II (Severely obese)	35	40
Obese Class III (Very severely obese)	40	

Source: WHO, 2006

These reference ranges are premised on the association between body weight, susceptible disease and death (WHO, 1995). The consequences of elevated level in adults means that those individuals picked as overweight and obese are at elevated risk for diseases such as hypertension, dyslipidaemia, coronary heart disease, stroke, type 2 diabetes mellitus, osteoarthritis among many others (*NHLBI, 1998*).

2.15 Serum Magnesium

Magnesium is an electrolyte which is often disregarded but is vital to life. Its use is seen in many enzymatic reactions. Magnesium is recognised to be key in the following processes: energy metabolism, utilization of glucose, protein synthesis, synthesis and breakdown of fatty acid and almost all hormonal reactions. As a result of its association with sodium, potassium, and calcium, it is also involved in the maintenance of ionic balance in the cells (Gums, 2004). It is a natural mineral found in diets that are rich in nuts, unpolished grains, and green vegetables (Song and Liu, 2012). Magnesium consumption is well-known to be insufficient in westernized populations (Ford & Mokdad, 2003; Shechter & Shechter, 2013).

Serum magnesium ion (Mg^{2+}) is considered the second most abundant intracellular cation and the fourth most common cation in the body (Ca^{2+}> K^{+}> Na^{+}> Mg^{2+}) (Laurant & Touyz, 2000). Almost 65% of the total Mg^{2+} concentration in the body is found in bone, about 34% found in the muscle, however 1% is found in the extracellular fluid (Drueke and Lacour, 2007). Magnesium ion (Mg^{2+}), be it intra and extracellular exists in 3 main states: (i) free or ionized (this is the physiologically active form); (ii) protein bound (this form bound to mostly cellular proteins and physiologically inactive); and (iii) form complexed to such anions like phosphate, citrate, bicarbonate and lactate). Of all the magnesium present in the extracellular fluid, 7% is

complexed to anions, 33% is protein bound and the remaining 60% exists in the physiologically ionized state (mostly bound to and transported by albumin) (Speich *et al.*,1981). Intracellular magnesium (Mg^{2+}) is found in the compartments of the following cell organelles:mitochondria, nuclei, and endo/sarcoplasmic reticulum (Romani *et al.*, 1993). In the nucleus and mitochodrion, magnesium binds to nucleic acid and chromatin, intermembrane proteins and matrix adenine phosphonucleotides respectively whilst it binds to ribonuclear proteins and phospholipids in the endo/sarcoplasmic reticulum (Romani, 2007). As a result of this bindingonly a minute fraction of intracellular magnesium occurs freely. The overall intracellular magnesium concentration spans between 14 and 20 x 10^4mmol/l, while the intracellular free magnesium concentration is projected to be about 0.5–0.7 x 10^6mmol/l (Romani *et al.*, 1993; Wolf *et al.*, 2003).

2.16 Magnesium Intake, Metabolism and Homeostasis

The blood level of magnesium is reliant on consumption of food and drinks rich in magnesium. The essential sources of dietary magnesium include green vegetables, legumes, whole seeds, unmilled grains and nuts. Fish, meat, and fruits have been known to also contain a good amount of magnesium. Hard water is another source of magnesium. Magnesium absorption is affected by several factors which either inhibit or enhance it. The presence of fibre, phytate, alcohol or excess amounts of phosphate and calcium can impede absorption of magnesium. Magnesium is usually lost from foods that undergo refining or processing (Saris *et al*., 2000; Shils & Shike; 2006). There is an uncertain effect of vitamin D on magnesium absorption. However, vitamin D and its active metabolites have been demonstrated to cause a rise in magnesium absorption in the intestines in normal humans and patients that have failure of the kidney (Shils & Shike; 2006). Reference limit of plasma concentration of serum magnesium lies within the range (0.75 to 0.95) mM (Weisinger & Bellorin-Font, 1998). Recommended daily consumption of magnesium in diet

is 350 mg and 280mg for males and females respectively. Nonetheless, during pregnancy, the daily requirement is increased to 355 mg. Research has revealed the consumption of magnesium in countries in the West to be currently lower than the value recommended (Saris *et al*., 2000).

Homeostasis of magnesium is reliant on the collective activities of the intestine, responsible for Mg^{2+} uptake from food, the bone, responsible for storing Mg^{2+} in its hydroxy-apatite form, and the kidneys, which regulates urinary Mg^{2+} excretion. The maintenance of plasma magnesium levels is dependent on its consumption, effectiveness of absorption by the intestines and renal system and also on renal excretion including several other pertinent factors including hormone in circulation. Two pathways, paracellular and transcellular pathways result in the absorption of Mg^{2+}. Paracellular pathway is an inactive mechanism which leads to the absorption of Mg^{2+} through tiny spaces between epithelial. The transcellular pathway consists of the movement of Mg^{2+} through the interior of epithelial cell to the blood. A maximum of about 40% of magnesium in the diet is absorbed via the intestine at the ileum and jejunum. The absorption of magnesium begins after 1 hour to 8 hours of ingestion and the ingested material reaches the large bowel in humans after 12 hours where there is no absorption or absorption is minimal (Shils & Shike, 2006; de Baaij *et al*. 2012).

By the processes of glomerular filtration and tubular reabsorption, the kidneys play a crucial role in regulating magnesium homeostasis. About 10 % (approx 100 mmol or 2400mg) of the total body magnesium undergoes glomerular filtration in healthy human and only 5% is lost in urine with the remaining being reabsorbed in different sections of nephron. Sites for the reabsorption of magnesium include the proximal tubule, distal convoluted tubule and the thick ascending loop of Henle (de Baaij *et al*. 2012).

2.17 Hypomagnesemia in CVD

The concentration of total magnesium present in our body may vary. Hypomagnesaemia shows reduction of body magnesium and it occurs when the serum magnesium concentration is less than 0.74mmol/l. Majority of hypomagnesaemia disorders come without any clear symptoms. Symptoms only occur when serum magnesium levels drops lower than 1.2mg/dl (Assadi, 2010). The overall prevalence of hypomagnesaemia is projected at 2% (Liamis *et al*., 2013). However it is thought to be as high as 53% in individuals within high-risk category which includes people suffering from chronic heart failure (Adamopoulos *et al*., 2009). Serum magnesium is still not measured frequently even though hypomagnesaemia may possibly have acute and chronic complications (de Baaij *et al.*, 2015).

Insufficiency in magnesium may be due to several causes and mechanisms which include: magnesium redistribution in the body; a drop in dietary intake as well as reduced absorption; diabetes mellitus; endocrine causes; renal loss; alcohol and drugs (Fawcett *et al*., 1999; Swaminathan, 2003). Hypomagnesaemia has been shown to be related to ventricular and atrial arrhythmias hence low serum magnesium could possibly be a risk factor for sudden cardiac death (Adamopoulos *et al*., 2009).

2.18 Causes of Hypomagnesemia in CVD

Magnesium redistribution: During certain conditions such as refeeding syndrome, magnesium moves from the extracellular fluid (ECF) to cells and bones of the body thus resulting in hypomagnesia. This redistribution of magnesium also occurs as a mechanism for correcting

acidosis. It is also seen in hungry bone syndrome, where patients have diffuse osteoblastic metastases (Swaminathan, 2003).

Gastrointestinal causes: During certain conditions such as malabsorption syndrome and chronic diarrhea, intestinal absorption of magnesium might be impaired resulting in reduced levels of serum magnesium (Swaminathan, 2003).

Renal loss: Loss of magnesium in urine might occur as a result of impairment in renal tubular reabsorption of magnesium. Excessive excretion of magnesium as seen in the use of diuretics may serve as an alternative cause of magnesium exhaustion from the loop of Henle being the key location for reabsorption of magnesium (Shils & Shike; 2006). Since tubular reabsorption of magnesium is related to sodium and calcium reabsorption, a fall in the reabsorption of sodium might result in magnesium loss (Weisenger & Bellorin-Font, 1998; Swaminathan, 2003). A number of disorders have been implicated in the reabsorption of magnesium by the kidneys and include the barters (Swaminathan, 2003).

Diabetes Mellitus: Diabetes is the most predominant disorder due to body magnesium insufficiency. Hyperglycosuria as seen in diabetes mellitus is known to cause loss of magnesium by the kidneys and osmotic diuresis which results in magnesium concentration lessening in the diabetes patient. Depletion in magnesium is known to disturb the secretion of insulin and result in insulin resistance. This observation may be as a result of unusual metabolism of blood glucose as magnesium is involved in the cycle. The action of tyrosine kinase at the insulin receptor may also be affected (Takaya *et al.*, 2004). Low blood level of magnesium is also linked to mortality due to type 2 diabetes mellitus (Dasgupta *et al.*, 2012).

Alcohol intake: Hypomagnesaemia is implicated in prolonged alcoholism and has an incidence rate of 29.8%. This may be linked to poor nutritional intake by alcoholics, vomiting, and diarrhea. Chronic pancreatitis due to alcoholism with malabsorption may also cause

hypomagnesaemia. Other causes of hypomagnesaemia in alcoholics include deficiency in vitamin D, dysfunction of the renal tubules, and the alteration in ionic permeability by ethanol (Stasiukyniene, 2002; Romani 2008). Hypomagnesaemia may result in a number of disorders like cardiovascular disease, diabetes, and osteoporosis. Predominant indicator of hypomagnesaemia is hypocalcaemia, cardiac arrhythmias, and elevated blood pressure (Swaminathan, 2003).

2.19 Hypermagnesaemia in CVD

This is the presence of high amounts of magnesium in body and may be associated with excessive consumption of magnesium containing diet or drugs containing magnesium that is frequently observed in people having kidney failure or impaired kidney function. Manifestation of hypermagnesaemia is very uncommon. However it could lead to several cardiovascular, neuromuscular defects and hypocalcaemia. Symptoms of hypermagnesaemia include nausea, headaches, vomiting, lethargy, and/or flushing.WhenMg^{2+} levels rise above 3.0mM, severe cardiac defects like hypotension and brachycardia occur. Extreme hypermagnesaemia can therefore result in cardiac arrest, then coma and death. However, hypermagnesaemia is unusual and until now has no genetic linkage. A markedly high body magnesium level may possibly lead to cardiotoxicity (Swaminathan, 2003). Strong clinical predictor for hypermagnesaemia is chronic kidney disease and patients on dialysis usually have raised magnesium concentration (Spiegel, 2011).

2.20 Serum calcium, HDL, LDL, Cholesterol and fasting blood sugar, PO_4 and CK in CVD

The level of HDL cholesterol may be influenced by serum calcium. Serum calcium has also been shown to decrease the level of serum LDL and total cholesterol (Reid *et al.,* 2002). Given that

dyslipidaemia is a principal predisposing factor for CHD, the association between calcium level of the body and CHD needs extensive study. Serum calcium has further been observed to rise with elevating levels of diastolic and systolic blood pressure (Kesteloot & Geboers, 1982); (Lind *et al.,* 1988). Also, in people having excessive level of parathyroid hormone in circulation and hence suffer from chronic hypercalcemia, hypertension is observed to be prevalent (Rosenthal & Roy, 1972) so that an elevated risk of mortality particularly from CVDs exists (Palmer *et al.,* 1987).

Serum calcium has also correlates with serum cholesterol and blood glucose and is therefore clearly related with the metabolic syndrome (Lind *et al.,* 1988).Some studies indicated that there was a significant positive correlation between triglyceride levels and serum calcium in the group with normal blood pressure and that LDL cholesterol showed a negative relationship with serum calcium in males but not in females (Vaskonen *et al.,* 2002). Sex and racial differences are believed to influence the relationship between serum calcium and blood lipids. It is believed that consumption of diets with high calcium content might be correlated with less global adiposity (Torres *et al.,* 2011).

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 Study design

This was a case-control study design with a convenience and randomised sampling technique conducted at the Department of Medicine and Therapeutics of the Korle Bu Teaching Hospital.

3.2 Study site

The sites include the Central Out-Patients Department (COPD), the Stroke unit and the Medical ward all under the Department of Medicine and Therapeutics of the Korle Bu Teaching Hospital. The Department of Medicine and Therapeutics attends to a broad range of health conditions including patients of cardiovascular diseases for both in- and out-patient settings. It has a unit dedicated to Stroke patients and a day for conducting outpatient clinic for patients with CVD conditions with adequate medical experts.

3.3 Study population

The cases were recruited from those on admission in the Medical wards and the stroke unit while the control subjects were recruited from the Central Out-Patients Department of the Hospital and members of staff of the Hospital. Both cases and controls were age and gender matched.

3.4 Inclusion criteria

Cases: The cases included all adult male or female patients aged between 18 and 80 years clinically diagnosed by a physician of having CVD condition such as congestive cardiac failure (CCF), Ischaemic heart disease (IHD), hypertensive heart disease (HHD) and Stroke also known as cerebrovascular accident (CVA).

Controls: Other adult patients aged between 18 and 80 years reporting at the OPD of the KBTH with medical conditions other than CVDs. No record of hypertension, myocardial infarction or stroke, dyslipidaemia, diabetes, liver, kidney diseases or metabolic syndromes certified by the physician on duty.

3.5 Exclusion criteria

Any subject that failed to meet the inclusion criteria above was excluded from participating in the study. Any participant unwilling to sign the consent form was excluded from participating in the study.

3.6 Sample size determination

The study adopted Kelsey's formula, (Kelsey *et al.*, 1996), to calculate the sample size. In a similar work done in Indian population by Dasgupta *et al*., (2012), the prevalence rate of hypomagnesaemia found among the case group was 11.9% and among control group was 9.8%. However, this study presumed a prevalence rate of 28.9% in the case group and 8.9% in control group. A ''ratio'' (r) of 1:1 is being kept between the control group and case group for the study. The study also maintains an 80% ''statistical power'' (Z_β) with 95% ''confidence level '' (Z_α) and an ''Odds Ratio'' (OR) of 2.74.

Thus;

Two-sided confidence level (1-alpha) (Z_{α})	95	1.96
Power(% chance of detecting) (Z_{β})	80	0.84
Ratio of Controls to Cases (r)	1:1	1
Hypothetical proportion of controls with exposure (P_2)	8.9%	0.089
Hypothetical proportion of cases with exposure (P_1)	28.9%	0.289
$\overline{P}$	(0.089+0.289)/2	0.189
Least extreme Odds Ratio to be detected (OR)	2.74	2.74

With the formula $$n = \left(\frac{r+1}{r}\right)\frac{(\bar{p})(1-\bar{p})(Z_{\beta}+Z_{\alpha/2})^2}{(p_1-p_2)^2}$$

$n_1 = \frac{(1+1)\,(0.189)\,(1-0.189)\,(0.84+1.96)^2}{1\qquad(0.289-0.089)^2}$

$n_1 = (2)\ \frac{(0.189)\,(0.811)\,(2.8)^2}{1\qquad(0.2)^2}$

$n_1 = (2)\,(0.189)\,(0.811)\,(2.8)^2 / 0.04$

$n_1 = (2)\,(1.202) / 0.04$

$n_1 = 60$

Since the study keeps 1:1 controls ratio, then the $n_1=n_2$. Thus, $N= n_1+n_2 = 120$.

3.7 Sampling Technique

A convenience and systematic sampling technique was employed in this study.

3.8 Subject Recruitment

Designed questionnaires were administered to gather basic demographic and CVD risk factor data of participants. Selected nurses and Laboratory Scientists were trained for the administration and collection of data and samples.

Upon case identification and referral by the physician, the investigator systematically approached the prospective participant and provided education on the importance of the research as contained in participant information sheet and sought their consent. However, in very ill but potential participants, this education and consent was directed at the close relative care-taker. Where consent was obtained, the questionnaire was administered and samples collected in accordance with study protocol. .

3.9 Demographics

The weight, sex, height, age and occupation of all the participants were obtained through the administration of questionnaires. The questionnaire for patient baseline demographics and clinical information was pretested with participants (10 cases and 10 controls) at the Department of Medicine and Therapeutics of the KBTH.

3.10 Blood collection

The method as described in the Standard Operating Procedure for performing venepuncture in the KBTH by Acquaye (1991) was used. Venous fasting blood samples were collected from the patients into labeled tubes (sodium fluoride and gel separator tubes). Rubber tourniquet was tied to the biceps about 8cm above the elbow joint for less than one minute and the site of puncture cleansed with methylated spirit. Then 5ml of blood was drawn from the brachial vein with a 19G hypodermic needle fixed on 5mls syringe. All aseptic conditions were adhered to. The blood

was immediately divided into two tubes; gel separated (4.0ml) for the general chemistry and sodium fluoride (1.0ml) for blood glucose measurement. The blood in the sodium fluoride tube was mixed to prevent clotting by gently inverting the tubes four times manually. Each tube was appropriately labeled with codes, sample type and time taken. The blood in the sodium fluoride tube was spun at a speed of 2000 RPM for 5 minutes while the blood in the gel separating tubes were allowed to clot before spinning at a speed of 3000 RPM for 10 minutes.

Each sample plasma and serum were drawn from the test tube with a micropipette into two labeled eppendorf tubes and stored at -20^0Cuntil required for use.

3.11 Laboratory Procedures

Serum Mg^{2+}, Ca^{2+}, K^+, PO_4, CK, Total Cholesterol and Fasting Plasma Glucose.

- Laboratory processing of the samples collected was carried out at the Central Laboratory Service of the Korle Bu Teaching Hospital using Dry Chemistry Analyzer VITRIOS 5.1 FS by OrthoClinicals-USA to run the following.
- Mg^{2+}, Ca^{2+}, K^+, PO_4, CK, total cholesterol and fasting plasma glucose.

3.11.1 Principle of the Procedure for VITROS Magnesium (Mg^{2+}) Slide test

The principle involves a colorimetric approach with approximate incubation time of 5 minutes at a 37 °C temperature environment using heparinised plasma or serum in a reaction volume of 5 µL in which a drop of patient sample is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers. Magnesium (both free and protein-bound) from the sample then reacts with the formazan dye derivative in the reagent layer; the high magnesium affinity of the dye dissociates magnesium from binding proteins. The resulting magnesium-dye complex causes a shift in the dye absorption maximum. The amount of dye complex formed is

proportional to the magnesium concentration present in the sample and is measured by reflection density at 630nm wavelength on VITROS 5,1 FS Chemistry Analyzer.

Reaction Scheme

Mg^{2+} + Ca^{2+} chelator Mg^{2+} + Ca^{2+}-chelator complex

Mg^{2+} + formazan dye derivative $\xrightarrow{\text{pH 9.75}}$ Mg^{2+}-dye complex

3.11.2 Principle of the Procedure for VITROS Calcium (Ca^{2+}) Slide test

The principle involves a colorimetric approach with approximate incubation time of 5 minutes at a 37 °C temperature environment using heparinised plasma or serum in a reaction volume of 10 µL. A drop of patient sample is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers. The bound calcium is dissociated from binding proteins, allowing the calcium to penetrate through the spreading layer into the underlying reagent layer. There, the calcium forms a complex with Arsenazo III dye, causing a shift in the absorption maximum. After incubation, the reflection density of the coloured complex is measured spectrophotometrically at 680nm wavelength on VITROS 5,1 FS Chemistry Analyzer. The amount of colored complex formed is proportional to the calcium concentration in the sample.

Reaction Scheme

Ca^{2+} + Arsenazo III $\xrightarrow{\text{pH 5.6}}$ colored complex

3.11.3 Principle of the Procedure for VITROS Phosphate (PO_4) Slide test

The principle involves a colorimetric method using heparinised plasma or serum in which a drop is placed on a multilayered, analytical element coated on a polyester support. The analytical principle is based on the reaction of inorganic phosphate with ammonium molybdate to form an ammonium phosphomolybdate complex at acidic pH, as described by Fiske and Subbarow

(1925). p-Methylaminophenol sulfate, an organic reductant reported by Gomori (1942), reduces the complex to form a stable heteropolymolybdenum blue chromophore. A drop of patient sample is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers. Phosphorus in the specimen forms a complex with ammonium molybdate. This complex is reduced by p methylaminophenol sulfate to give a blue complex. The concentration of phosphorus in the sample is determined by measuring the heteropolymolybdenum blue complex by reflectance spectrophotometry on VITROS 5,1 FS Chemistry Analyzer.

Reaction Scheme

Inorganic phosphate + ammonium molybdate $\xrightarrow{\text{pH 4.2}}$ ammonium phosphomolybdate complex

ammonium molybdate complex $\xrightarrow{\text{p-methylaminophenol}}$ sulphate heteropolymolybdate blue

3.11.4 Principle of the Procedure for VITROS Creatine Kinase (CK) Slide test

The principle of the method is based on an enzymatic multilayered rate approach with approximate incubation time of 5 minutes at a 37 °C temperature environment using heparinised plasma or serum. A drop of patient sample is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers. This layer also contains N-acetylcysteine (NAC) to activate CK without pretreating the sample. When the sample is deposited on the slide, creatine kinase catalyzes the conversion of creatine phosphate and ADP to creatine and ATP. In the presence of glycerol kinase (GK), glycerol is phosphorylated to L-α-glycerophosphate by ATP. Oxidation of L-α-glycerophosphate to dihydroxyacetone phosphate and hydrogen peroxide occurs in the presence of L-α-glycerophosphate oxidase (α-GPO). Finally, leuco dye is oxidized by hydrogen peroxide in the presence of peroxidase to form a dye. Reflection densities are monitored during incubation. The rate of change in reflection density is then converted to enzyme activity measured at 670nm wavelength on VITROS 5,1 FS Chemistry Analyzer.

Reaction Scheme

creatine phosphate + ADP $\xrightarrow{CK,\ NAC,\ Mg^{2+}}$ creatine + ATP

glycerol + ATP $\xrightarrow{GK}$ L-α-glycerophosphate + ADP

L-α-glycerophosphate + O_2 $\xrightarrow{\alpha\text{-GPO}}$ dihydroxyacetone phosphate + H_2O_2

H_2O_2 + leuco dye $\xrightarrow{peroxidase}$ dye + $2H_2O$

3.11.5 Principle of the Procedure for VITROS Albumin (ALB) Slide test

The principle of the method is a colorimetric based approach with approximate incubation time of 2.5 minutes at a 37 °C temperature environment using heparinised plasma or serum in a reaction volume of 5.5 µL. A drop of patient sample is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers. When the fluid penetrates the reagent layer, the bromcresol green (BCG) dye diffuses to the spreading layer and binds to albumin from the sample. This binding results in a shift in wavelength of the reflectance maximum of the free dye. The color complex that forms is measured by reflectance spectrophotometry measured at 630nm wavelength on VITROS 5,1 FS Chemistry Analyzer. The amount of albumin-bound dye is proportional to the concentration of albumin in the sample.

Reaction Scheme

albumin + bromcresol green (BCG) $\longrightarrow$ BCG-albumin complex

3.11.6 Principle of the Procedure for VITROS Potassium (K^+) Slide test

This method uses a potentiometric principled approach with approximate incubation time of 2 minutes at a 37 °C temperature environment using heparinised plasma or serum in a reaction and reference volumes of 10 µL. This is a multilayered, analytical element coated on a polyester support that uses direct potentiometry for measurement of ionic potassium on VITROS 5,1 FS

Chemistry Analyzer. The slide consists of two ion-selective electrodes, each containing valinomycin (an ionophore for potassium), a reference layer, and a silver chloride layer coated on a polyester support. A drop of patient sample and a drop of VITROS Reference Fluid on separate halves of the slide results in migration of both fluids toward the center of the paper bridge. A stable liquid junction is formed connecting the reference electrode to the sample indicator electrode. Each electrode produces an electrical potential in response to the activity of potassium applied to it. The potential difference poised between the two electrodes is proportional to the potassium concentration in the sample.

3.11.7 Principle of the Procedure for Fasting Blood Glucose (GLU) Slide test

The method is a colorimetric approach based principle with approximate incubation time of 5 minutes at a 37 °C temperature environment using heparinised plasma or serum in a reaction volume of 10 µL. A drop of patient sample is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers. The oxidation of sample glucose is catalyzed by glucose oxidase to form hydrogen peroxide and gluconate. This reaction is followed by an oxidative coupling catalyzed by peroxidase in the presence of dye precursors to produce a dye. The intensity of the dye is measured by reflected light measured at 540nm wavelength on VITROS 5,1 FS Chemistry Analyzer. The dye system used is closely related to that first reported by Trinder (1969). The chemistry of the glucose slides has been described by Curme and Rand (1997).

Reaction Scheme

β-D-glucose + O_2 + H_2O $\xrightarrow{\text{glucose oxidase}}$ D-gluconic acid + H_2O_2

$2H_2O_2$ + 4-aminoantipyrine + 1,7-dihydroxynaphthalene $\xrightarrow{\text{peroxidase}}$ red dye

3.11.8 Principle of the Procedure for VITROS Cholesterol (CHOL) Slide test

The principle of the method involves a colorimetric approach with approximate incubation time of 5 minutes at a 37 °C temperature environment using heparinised plasma or serum in a reaction volume of 5.5 µL and measured at 540nm wavelength. The method is based on an enzymatic method similar to that proposed by Allain *et al*. (1974). A drop of patient sample is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers. The Triton X-100 (TX100) surfactant in the spreading layer aids in dissociating the cholesterol and cholesterol esters from lipoprotein complexes present in the sample. Hydrolysis of the cholesterol esters to cholesterol is catalyzed by cholesterol ester hydrolase. Free cholesterol is then oxidized in the presence of cholesterol oxidase to form cholestenone and hydrogen peroxide. Finally, hydrogen peroxide oxidizes a leuco dye in the presence of peroxidase to generate a colored dye. The density of dye formed is proportional to the cholesterol concentration present in the sample and is measured by reflectance spectrophotometry on VITROS 5,1 FS Chemistry Analyzer.

Reaction Scheme

Lipoprotein $\xrightarrow{\text{TX100}}$ cholesterol + cholesterol esters + proteins

Cholesterol esters + H_2O $\xrightarrow{\text{cholesterol ester hydrolase}}$ Cholesterol + fatty acids

Cholesterol + O_2 $\xrightarrow{\text{cholesterol oxidase}}$ cholest-4-en-3-one + H_2O_2

H_2O_2 + leuco dye $\xrightarrow{\text{peroxidase}}$ dye + $2H_2O$

3.11.9 Principle of the Procedure for VITROS Triglycerides (TRG) Slide test

The principle of the method is a colorimetric approach with approximate incubation time of 5 minutes at a 37 °C temperature environment using heparinised plasma or serum in a reaction volume of 5.5 µL and measured at 540nm wavelength. The analysis is based on an enzymatic method as described by Spayd *et al*. (1978). A drop of patient sample is deposited on the slide

and is evenly distributed by the spreading layer to the underlying layers. The Triton X-100 surfactant in the spreading layer aids in dissociating the triglycerides from lipoprotein complexes present in the sample. The triglyceride molecules are then hydrolyzed by lipase to yield glycerol and fatty acids. Glycerol diffuses to the reagent layer, where it is phosphorylated by glycerol kinase in the presence of adenosine triphosphate (ATP). In the presence of L-α-glycerol-phosphate oxidase, L-α-glycerophosphate is then oxidized to dihydroxyacetone phosphate and hydrogen peroxide. The final reaction involves the oxidation of a leuco dye by hydrogen peroxide, catalyzed by peroxidase, to produce a dye. The density of the dye formed is proportional to the triglyceride concentration present in the sample and is measured by reflectance spectrophotometry on VITROS 5,1 FS Chemistry Analyzer.

Reaction Scheme

Lipoproteins $\xrightarrow{\text{surfactant}}$ triglycerides + proteins

Triglycerides + H_2O $\xrightarrow{\text{lipase}}$ glycerol + fatty acids

Glycerol + ATP $\xrightarrow{\text{glycerol kinase + } MgCl_2}$ L-α-glycerophosphate + ADP

L-α-glycerophosphate + O_2 $\xrightarrow{\text{L-α-glycerol-phosphate oxidase}}$ dihydroxyacetone phosphate + H_2O_2 + leuco dye $\xrightarrow{\text{peroxidase}}$ dye + $2H_2O$

3.11.10 Principle of the Procedure for VITROS High Density Lipoprotein (HDL) Slide test

The method uses a colorimetric approach with approximate incubation time of 5 minutes at a 37 °C temperature environment using heparinised plasma or serum in a reaction volume of 10 µL and measured at 670nm wavelength. The method is based on a non-HDL precipitation method similar to one used by Burstein *et al*. (1970) followed by an enzymatic detection similar to that proposed by Allain *et al*. (1974). A drop of patient sample is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers. HDL is separated by the precipitation

of non- High Density Lipoproteins (non-HDL) using phosphotungstic acid (PTA) and magnesium chloride (MgCl2) in the spreading layer. The Emulgen B-66 surfactant in the spreading layer aids in the selective dissociation of the cholesterol and cholesterol esters from the HDL lipoprotein complexes present in the sample. Hydrolysis of the HDL-derived cholesterol ester to cholesterol is catalyzed by a selective cholesterol ester hydrolase. Free cholesterol is then oxidized in the presence of cholesterol oxidase to form cholestenone and hydrogen peroxide. Finally, hydrogen peroxide oxidizes a leuco dye in the presence of peroxidase to generate a colored dye. The density of dye formed is proportional to the HDL cholesterol concentration present in the sample and is measured by reflectance spectrophotometry on VITROS 5,1 FS Chemistry Analyzer.

Reaction Scheme

HDL + non-HDL $\xrightarrow{PTA/MgCl_2}$ high density lipoproteins + non-HDL(↓)

High density lipoproteins $\xrightarrow{Emulgen\ B\text{-}66}$ cholesterol + HDL cholesterol esters +proteins

HDL cholesterol esters + H_2O $\xrightarrow{cholesterol\ ester\ hydrolase}$ cholesterol and fatty acids

cholesterol + O_2 $\xrightarrow{cholesterol\ oxidase}$ cholest-4-en-3 + H_2O_2

H_2O_2 + leuco dye $\xrightarrow{peroxidase}$ dye + H_2O

3.12 Data Analysis

Data analysis was done with the Statistical Package for the Social Sciences (SPSS) software package version 20.0. The Student *t*-test for unpaired data was used to test for any statistical difference between two means of the cases and controls. The correlation co-efficient (r) was used to show the strength and direction of the association between the two linear variables. The co-efficient determination (r^2) was used to show the degree to which the measured variables are accounted for or explained by the detected observations. Pearson correlation coefficient was

done to test linear relationship between and within two clinical variables. A crosstab was performed to test relationship between and among at least three different clinical variables. Linear regression analysis was performed to test cause and effect relationship with or without CVD types. The odds ratio within study population was also determined. Univariate analysis was performed to test the hypothesis of magnesium effect on CVD types restricting all confounding factors. A p-value of ≤ 0.05 will be considered significant with a confidence interval of 95%.

CHAPTER FOUR

4.0 RESULTS

4.1 Study Population Demographics and Characteristics

A total of one hundred and twenty-eight (128) participants were recruited for the study. The cases and controls were made up of comparable males/females and number. The mean ages for the cases and controls were also comparable. The participants included sixty (60) cases and 68 controls. The cases were made up of 29 males and 31 females, whereas the controls were made up of 35 males and 33 females (Table 4.1). The mean ages for the cases and controls were 58.35±13.76 and 53.44±16.37 years respectively and not statistically significant ($p = 0.0860$). About 73% and 56% of both the cases and controls were aged between 48 and 77 years respectively. On educational status of participants, thirty-five percent (35%) of cases had secondary education compared to 25% of the control. Majority of the cases were non-smokers (95%); however 35% took in alcohol. On marital status, 62% of the controls and 63% of the cases were married. Majority of the participants were of Christian religion of which controls formed 88.24% and 95% constituted the cases. Again, the majority of the participants were in employment of which controls and cases respectively constituted about 53% and 48%. Less than 50% of all categories of participants undertake one form of exercise or the other. Of meal type preference, majority of study participants regularly take a local meal, banku and okro.

Table 0:1Distribution of general characteristics and demographics of the study population

Parameters	Total (n=128)		Male (n=64)		Female (64)	
	Control (n=68)	Case (n=60)	Control (n=35)	Case (n=29)	Control (n=33)	Case (n=31)
Gender						
Male	35(51.47)*	29(48.33)				
Female	33(48.53)	31(51.67)				
Marital Status						
Single	14(20.59)	2(3.33)	9(25.71)	2(6.90)	5(15.15)	0(0.00)
Married	42(61.76)	38(63.33)	23(65.71)	23(79.31)	19(57.58)	15(48.39)
Divorced	1(1.47)	9(15.00)	1(2.86)	4(13.79)	0(0.00)	5(16.13)
Widowed	11(16.18)	11(18.33)	2(5.71)	0(0.00)	9(27.27)	11(35.48)
Religion						
Christian	60(88.24)	57(95.00)	31(88.57)	26(89.66)	29(87.88)	31(100.00)
Muslims	8(11.76)	3(5.00)	4(11.43)	3(10.34)	4(12.12)	0(0.00)
Education						
No Formal Edu.	2(2.94)	7(11.67)	1(2.86)	2(6.90)	1(3.03)	5(16.13)
Basic Edu.	19(27.94)	20(33.33)	7(20.00)	11(37.93)	12(36.36)	9(29.03)
SHS/O-Level	17(25.00)	21(35.00)	8(22.86)	13(44.83)	9(27.27)	8(25.81)
HND/Diploma	11(16.18)	5(8.33)	7(20.00)	1(3.45)	4(12.12)	4(12.90)
Bachelor	14(20.59)	7(11.67)	9(25.71)	2(6.90)	5(15.15)	5(16.13)
Postgraduate	5(7.35)	0(0.00)	3(8.57)	0(0.00)	2(6.06)	0(0.00)
Employment						
Student	3(4.41)	1(1.67)	3(8.57)	1(3.45)	0(0.00)	0(0.00)
Unemployed	5(7.35)	10(16.67)	2(5.71)	2(6.90)	3(9.09)	8(25.81)
Employed	36(52.94)	29(48.33)	20(57.14)	19(65.52)	16(48.48)	10(32.26)
Retired	24(35.29)	20(33.33)	10(28.57)	7(24.14)	14(42.42)	13(41.94)
Smoking	0(0.00)	3(5.00)	0(0.00)	3(10.34)	0(0.00)	0(0.00)
Alcohol	14(20.59)	21(35.00)	14(40.00)	14(48.28)	0(0.00)	7(22.58)
Exercise	33(48.53)	19(31.67)	15(42.86)	8(27.59)	18(54.55)	11(35.48)
Age						
18 -27 yrs	6 (8.82)	2 (3.33)	4 (11.43)	2 (6.90)	2 (6.06)	0 (0.00)
28 – 37 yrs	6 (8.82)	2 (3.33)	2 (5.71)	0 (0.00)	4 (12.12)	2 (6.45)
38 – 47 yrs	14 (20.59)	6 (10.00)	9 (25.71)	4 (13.79)	5 (31.03)	2 (6.45)
48 – 57 yrs	12 (17.65)	14 (23.33)	9 (25.71)	9 (31.03)	9 (15.15)	5 (16.13)
58 – 67 yrs	12 (17.65)	21 (35.00)	3 (8.57)	9 (31.03)	3 (9.09)	12 (38.71)
68 – 77 yrs	14 (20.59)	9 (15.00)	6 (17.14)	3 (10.34)	8 (24.24)	6 (19.35)
78 – 87 yrs	4 (5.88)	6 (10.00)	2 (5.71)	2 (6.90)	2 (6.06)	4 (12.90)

*Number (%)

4.2 A comparison of the clinical and biochemical variables between cases and controls of the study population

Comparing the mean values of the clinical and related biochemical parameters of the cases and controls of the study participants by Students t-test of unpaired data, the mean serum magnesium level was significantly lower (0.82±0.16) in the CVD patients as compared to the control (0.87±0.07) ($p = 0.0210$). The mean serum albumin was significantly lower in the CVD patients (39.10±6.69 g/L) as compared to the control (42.49±3.34 g/L) ($p = 0.0003$). However, the mean serum potassium was significantly higher in CVD patients (4.95±1.82 mmol/L) as compared to the controls (4.31±0.69 mmol/L) ($p = 0.0081$). The mean values of total cholesterol (4.77±1.51 mmol/L), HDL (1.22±0.48 mmol/L) and LDL (2.97±1.30 mmol/L) were significantly lower in the CVD patients as compared ~~to~~ with the control group (5.32±1.33 mmol/L, 1.38±0.31 mmol/L and 3.43±1.01 mmol/L) with respective p-values of 0.0303, 0.0252 and 0.0263. (Table 4.2).

Table 0:2 Comparison of clinical and biochemical variables between cases and controls of the study population

Parameters	Units	Cases (n = 60) Mean±SD	Controls (n = 68) Mean±SD	P-values
Age	years	58.35±13.76	53.69±16.37	0.0860
Mean SBP	mmHg	137.33±24.24	134.74±19.25	0.5022
Mean DBP	mmHg	85.83±13.25	81.22±14.00	0.0589
BMI	Kg/m^2	27.29±5.79	28.79±4.88	0.1143
Mg^{2+}	mmol/L	0.82±0.16	0.87±0.07	**0.0210**
Ca^{2+}	mmol/L	2.31±0.24	2.37±0.14	0.0822
ALB	g/L	39.10±6.69	42.49±3.34	**0.0003**
Adj. Ca^{2+}	mmol/L	2.33±0.19	2.30±0.18	0.3610
K^{+}	mmol/L	4.95±1.82	4.31±0.69	**0.0081**
PO_4^{2-}	mmol/L	1.32±0.31	1.40±0.19	**0.0050**
CK	mmol/L	238.45±304.08	220.28±219.06	**0.0107**
Tchol	mmol/L	4.77±1.51	5.32±1.33	**0.0303**
TG	mmol/L	1.17±0.73	1.45±0.93	0.0629
HDL	mmol/L	1.22±0.48	1.38±0.31	**0.0252**
LDL	mmol/L	2.97±1.30	3.43±1.01	**0.0263**
VLDL	mmol/L	0.57±0.33	0.70±0.54	0.1084
FBG	mmol/L	6.45±3.00	6.03±3.94	**0.0256**
Ca^{2+}/ Mg^{2+}		2.84±0.69	2.76±0.25	0.3739

SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; BMI: Basal Metabolic Index; Mg^{2+}: Magnesium ion; Ca^{2+}: Calcium ion; ALB: Albumin; Adj. Ca^{2+}: Adjusted Calcium ion; K^{+}: Potassium ion; PO_4^{2-}: Phosphate ion; CK: Creatine Kinase; Tchol: Total Cholesterol; TG: Triglycerides; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein; VLDL: Very Low Density Lipoprotein; FBG: Fasting Blood Glucose. Significance at **p< 0.05**

4.3 Cross tabulation

All observations were assumed to be independent in the cross tabulation analysis. This analysis showed the parametersthat had strong evidence against the null hypothesis (Table 4.3).The null hypothesis was:No difference between serum magnesium levels and related biochemical parameters. This means that by relating the analysis of Chi square to the likelihood ratio of the cardiovascular diseases, a stronger relationship was observed.

Table 0:3Cross Tabulation showing significant p-values

Crosstab	p-value(s)		
	Chi Square	Likelihood Ratio	Linear by Linear Assoc.
Age_{cat} x BMI_{cat} x $Mg^{2+}_{cat\ total}$	**0.026**	**0.017**	**0.051**
ALB_{cat} x BMI_{cat} x Mg^{2+}_{cat1}	**0.029**	**0.023**	0.920
FBG_{cat} x Age_{cat} x $Mg^{2+}_{cat\ total}$	**0.040**	**0.033**	**0.006**
Tchol x Gender x Mg^{2+}_{cat}	**0.035**	**0.032**	-
Age x Gender x Mg^{2+}_{cat2}	**0.022**	**0.019**	-
Age x Gender x $Mg^{2+}_{cat\ total}$	**0.035**	**0.030**	-
Adj. Ca^{2+} x DBP x Mg^{2+}_{cat2}	**0.011**	**0.010**	**0.001**
Adj. Ca^{2+} x DBP x $Mg^{2+}_{cat\ total}$	**0.038**	**0.015**	**0.037**
HDL_{cat} x DBP_{cat} x Mg^{2+}_{cat2}	**0.026**	**0.033**	**0.048**

Categories mean the parameter has been stratified: Mg^{2+}_{cat}as low = below reference range (< 0.70 mmol/L), normal = within reference range (0.70 – 1.00 mmol/L) and high = above reference range (> 1.00 mmol/L). BMI_{cat} was stratified as underweight (16 – 18.5 Kg/m^2), normal weight (18.5 – 25 Kg/m^2), overweight (25 – 30 Kg/m^2), moderately obese (30 – 35 Kg/m^2) and severely obese (35 – 40 Kg/m^2). Age was stratified in the range of ten years as (18 – 27), (28 – 37), (38 – 47), (48 – 57), (58 – 67), (68 – 77) and (78 – 87). ALB_{cat} was categorized as low = below reference range (< 35 g/L), normal = within reference range (35 – 50 g/L) and high = above reference range (> 50 g/L). FBG_{cat} was categorized as low = below reference range (< 4.1mmol/L), normal = within reference range (4.1 – 5.9mmol/L) and high = above reference range (>5.9mmol//L). HDL_{cat} was categorized as low = below reference range (< 1.03 mmol/L), normal = within reference range (1.03 – 1.55 mmol/L) and high = above reference range (> 1.55mmol//L). DBP_{cat} was categorized as low = below reference range (< 70 mmHg), normal = within reference range (70 – 80 mmHg) and high = above reference range (> 80 mmHg). Significance at **p< 0.05**

4.4 Mg^{2+} stratification of the study population

About 13% increase of CVD prevalence occurred in patient with low serum magnesium ions (Mg^{2+}) unlike those with high serum magnesium ions (Mg^{2+}). Magnesium (Mg^{2+}) stratification (Table 4.5) showed a significant influence on mean systolic and diastolic blood pressures, basal metabolic index, albumin, potassium, total cholesterol and low density lipoprotein.

Table 0:4**Clinical variables stratified by Mg^{2+}categories**

	LowMg^{2+}		NormalMg^{2+}			HighMg^{2+}		
Parameters of study	Cases (n = 8)	*Controls (n = 1)	Cases (n = 48)	Controls N = (65)	P-value	Cases (n = 4)	Controls (n = 2)	P-value
Age	59.45 ±12.5	61	58.20±11.95	53.44±16.37	0.0908	58.25±23.99	52.34±11.31	0.7671
Mean SBP	137.75±18.4	130	139.28±25.9	129.93±19.2	**0.0297**	120±7.79	129.37±1.41	0.1859
MeanDBP	86.08±14.1	82	87.08±13.10	80.23±14.00	**0.0094**	83.5±17.29	79.78±2.12	0.7890
BMI	26.42±6.5	30	26.35±4.84	28.31±4.88	**0.0364**	23.40±1.63	28.23±0.49	**0.0176**
Ca^{2+}	2.30±0.5	2.34	2.33±0.14	2.37±0.14	0.1361	2.39±0.11	2.38±0.03	0.9105
ALB	38.82±8.7	41	39.15±6.35	42.34±3.34	**0.0008**	41.75±2.36	42.45±1.41	0.7275
Adj. Ca^{2+}	2.32±0.4	2.32	2.34±0.12	2.31±0.18	0.3188	2.35±0.06	2.31±0.06	0.4844
K^{+}	4.99±1.3	3.5	5.12±2.06	4.29±0.69	**0.0030**	5.15±1.42	4.22±0.14	0.4325
PO_4^{2-}	1.31±0.3	1.35	1.28±0.22	1.32±0.19	0.3033	1.70±0.81	1.34±0.02	0.5854
**CK	221.2±423.4	139	219.2±302.9	200.2±219.1	0.7003	192.5±168.4	205.2±448.3	0.9590
Tchol	4.56±1.23	4.39	4.65±1.28	5.23±1.33	**0.0217**	4.53±1.07	5.21±1.44	0.5401
TRL	1.10±0.50	1.11	1.07±0.78	1.33±0.93	0.1190	1.04±0.25	1.30±0.81	0.5490
HDL	1.22±0.35	1.28	1.29±0.52	1.34±0.31	0.5252	1.02±0.21	1.34±0.24	0.1652
LDL	2.80±1.08	2.60	2.81±1.13	3.42±1.01	**0.0032**	3.04±0.95	3.43±1.32	0.6914
VLDL	0.50±0.23	0.51	0.49±0.36	0.64±0.54	0.0979	0.48±0.11	0.63±0.36	0.4430
FBG	6.50±2.48	15.07	6.57±3.45	6.18±3.94	0.5849	5.20±0.95	6.16±14.35	0.8854

*The p-value could not be determined for the low Mg^{2+} because only one of the control subject recorded a low value.Magnesium ion categories mean the parameter has been stratified as low = below reference range (< 0.70 mmol/L), normal = within reference range (0.70 – 1.00 mmol/L) and high = above reference range (> 1.00 mmol/L).Significance at **p< 0.05**

There were some outliers within the CK values of both the cases and controls thereby driving the **SD figures higher than expected.

4.5 Association between Magnesium and biochemical variables in the study population

The Pearson's Product Moment Correlation test revealed a significantly positive association between serum magnesium level with Ca, ALB, K^+,PO_4^{2-} and HDL, r = 0.264, 0.220, 0.216, 0.187, 0.184 (Table 4.5).

Table 0:5**Association between Magnesium and biochemical variables in cases**

Correlates	Pearson Correlation Coefficient (r)	Pearson Coefficient determination (r^2)	P- value (two tailed)
Mg^{2+} and Ca^{2+}	0.264	0.0696	**0.003**
Mg^{2+} and ALB	0.220	**0.0484**	**0.013**
Mg^{2+} and HDL	0.184	**0.0339**	**0.038**
Mg^{2+} and PO_4^{2-}	0.187	**0.0350**	**0.034**
Mg^{2+} and K^+	0.216	**0.0467**	0.077

Significance at **p< 0.05**

4.6 Association between Magnesium abnormality and cardiovascular disease

Magnesium abnormalities (both low and high) were positively associated with CVD in Table 4.6. Even though the trend of higher odds of outcome persisted when the exposed group was treated as those having only high magnesium levels, the odds ratio was not statistically significant (Table 4.6.1).

Table 0:6**Odds ratio showing Magnesium abnormality (both high and low levels) and cardiovascular disease**

Unadjusted Odds	CVD Cases (+)	Controls (-)	Odds Ratio =5.4167 (1.4484 – 20.2569) Yates X2 = 6.06, **p-value: 0.0138**; Corrected for continuity.
Magnesium ion (Mg2+) Abnormal (High or Low)	12	3	Fisher exact test two tail **p-value = 0.0111**
Magnesium ion (Mg2+) Normal	48	65	

Significance at **p< 0.05**

Table 0:6.1Odds ratio of abnormal Mg (high) in the study population

	Exposed	Unexposed	Total	Proportion Exposed
Cases	3	57	60	0.0500
Controls	2	66	68	0.0294
Total	5	123	1280.0391	
	Point Estimate		[95% confidence Interval]	
Odds Ratio	1.736842		0.1911334	21.38132 (exact)
Attr. frac. ex.	0.4242424		-4.231948	0.953230 (exact)
Attr. frac. pop	0.0212121			
	Chi^2 (1) = 0.36		Pr>Chi^2 = 0.5485	

Unexposed had both low and normal Mg levels; whilst exposed had only high Mg levels

4.7 Adjusted Odds Ratio (OR)

With an adjusted OR from binary logistics (table 4.7) and confounding variables e.g. SBP, BMI controlled and an adjustment made as SBP increment of 10 plus a BMI increment of 5; Mg^{2+} categories were seen to be associated with phosphate categories, adjusted calcium, CK categories, DBP categories, ALB categories and Tchol categories.

Table 0:7Adjusted odds ratio (binary logistics)

	Adjusted odds by binary logistic regression**		**Parameters**
A*	Controls (n = 47)	0.030 (-4.210 to -2.340); **p-value = 0.021**	Covariate: SBP 10, BMI 5, $PO_4^{2-}{}_{Cat}$
	Cases (n = 59)	0.073 (-4.060 to -1.940); **p-value = 0.017**	Dependent: $Mg^{2+}{}_{new\ cat}$
B	Cases (n = 59)	0.074 (-4.043 to -1.920); **p-value = 0.017**	Covariate: SBP 10, BMI 5, Adj.$Ca^{2+}{}_{Cat}$
	Controls (n = 52)	0.030 (-4.205 to -2.534); **p-value = 0.019**	Dependent: $Mg^{2+}{}_{new\ cat}$
C	Cases (n = 59)	0.073 (-4.060 to -1.852); **p-value = 0.016**	Covariate: SBP 10, BMI 5, $K^{+}{}_{Cat}$
	Controls (n = 52)	0.030 (-4.205 to -2.587); **p-value = 0.021**	Dependent: $Mg^{2+}{}_{new\ cat}$

*CK categories, DBP categories, ALB categories and Tchol categories gave the same odds and p-value but confidence interval was different. CK categories (CI=-4.060 to -1.658); DBP categories (CI = -4.060 to -2.005); ALB categories (CI = -4.205 to -2.408. Tchol (CI= -4.174 to -2.501). Significance at **p< 0.05**

**Adjusted odds controlled significant confounders from *section 4.6; however*, SBP and BMI were found to be significant confounders when OR was adjusted.

4.8 Linear regression analysis

This analysis was performed to test the hypothesis about the cause and effect relationship (s) that existed between magnesium and other biochemical parameters (Table 4.8) in the study with or without CVD types, having observed the trend in section 4.6 to 4.7.

Table 0:8 Linear Regression

Parameters	With Rule (CVD cases)	Without Rule
Dependent variable: $Mg^{2+}{}_{cat}$ Independent variable categories: Age, SBP, DBP, BMI, Adj. Ca^{2+}, K^{+}, PO_4^{2-}, CK, ALB, TRL, HDL, LDL, VLDL, FBG.	P = **0.013**	P = **0.013**

SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; BMI: Basal Metabolic Index; Mg^{2+}: Magnesium ion; Ca^{2+}: Calcium ion; ALB: Albumin; Adj. Ca^{2+}: Adjusted Calcium ion; K^{+}: Potassium ion; PO_4^{2-}: Phosphate ion; CK: Creatine Kinase; Tchol: Total Cholesterol; TG: Triglycerides; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein; VLDL: Very Low Density Lipoprotein; FBG: Fasting Blood Glucose. Significance at **p< 0.05**
CVD = cardiovascular type. **With rule**: CVD cases selected; **without rule**: no preference made

4.9 Univariate analysis

This analysis in Table 4.9 restricted and accounted for confounding variables thereby taking Mg^{2+} only and summarizing its influence on CVD having observed the trend in section 4.8.

Table 0:9Univariate Analysis

Parameters	Hypothesis Testing
Dependent variable: Mg^{2+}	
Fixed factor variable: Age_{cat} and $Gender_{cat}$	F_{df}, $\alpha = 8.069$
Covariate variable: CVD Types	
Random factor variable: Age, SBP, DBP, BMI, Adj. Ca^{2+}, K^{+}, PO_4^{2-}, CK, ALB, TG, HDL, LDL, VLDL, FBG.	P = **0.001**

SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; BMI: Basal Metabolic Index; Mg^{2+}: Magnesium ion; Ca^{2+}: Calcium ion; ALB: Albumin; Adj. Ca^{2+}: Adjusted Calcium ion; K^{+}: Potassium ion; PO_4^{2-}: Phosphate ion; CK: Creatine Kinase; Tchol: Total Cholesterol; TG: Triglycerides; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein; VLDL: Very Low Density Lipoprotein; FBG: Fasting Blood Glucose. Significance at **p< 0.05**

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 DISCUSSIONS

5.1.1 Serum Magnesium levels and CVDs

Magnesium is a typical macro element that is associated with cardiovascular related diseases (Ishimura *et al*., 2007; Lee *et al*., 2005; Kieboom *et al*., 2016) but this conflicts other studies (Khan *et al*., 2010). In this study, a report on baseline information of serum magnesium in the studied Ghanaian cardiovascular disease population and its impact on clinical and biochemical variables showed that a 13.33% increase risk of CVD prevalence in the participants with low magnesium was consistent with similar studies (Kieboom *et al.,* 2016). Notwithstanding, meta-analysis elsewhere (Ford, 1999 & Reunanen, 1996) found an increased risk of cardiovascular diseases (Bernardin *et al.,* 2005). Ford, (1999) used hazard ratio and found magnesium concentrations to have an indirect relationship with all-cause mortality and mortality occasioned by ischemic heart disease. Although studies reported by Reunanen (1996) found raised level of serum copper and low level of serum zinc to be related to elevated cardiovascular mortality, no such relationship was found with serum calcium and magnesium and associated mortality predisposition. Nevertheless, the current research work rather shared similar thoughts with Kieboom *et al.,* (2016) depicting serum magnesium levels to be considerably reduced in CVDs in comparison to healthy controls. This results was also consistent with several studies (Alexander *et al.,* 2008; Spiegel, 2011; Covic, 2010; Kanbay, 2010) especially among women and the elderly (Whang, 1987; Touitou *et al*., 1987). Low magnesium levels have been shown to cause damage to the endothelium and thereby could lead to cardiovascular diseases since magnesium promotes vasodilation, reduced vascular resistance, and lower systemic and coronary

blood flow and pressure (Baker *et al.*, 2009) as it generates a pro-inflammatory, prothrombotic, and pro-atherogenic environment that might lead to the onset of CVD (Maier *et al.*, 2004). In myocardial tissues, the ability of magnesium to antagonize calcium activity during ischaemia limits infarct size; reduces coronary artery spasm, and limits post-infarction oxidative damage (Leor, 1995). Magnesium deficiency was reported to raise blood pressure in animal models and its supplementation had led to the avoidance of raised blood pressure.

All CVD types of interest e.g. CCF, IHD, HHD and Stroke had decreased magnesium levels in this study. The benefits of magnesium in cardiovascular patients include opposing release of glutamate, blockade of n-methyl d-aspartate receptor, resistive action of calcium channel, and maintenance of blood flow to the cerebral region (Muir, 2002). Even though the classification of cardiovascular mortality is heterogenic (Kieboom *et al.* 2016) with an inclusion of non - atherosclerotic outcomes and the heterogeneity often made it difficult to study associations underlying CVD pathophysiologies, the study was able to use all CVD types of congestive cardiac failure, hypertensive heart disease, cerebrovascular accident (Stroke) and ischaemic heart disease with the help of linear regression and univariate analytical tools. In this study, serum magnesium levels were significantly associated with or without CVD as the preferred option in our model ($p = 0.013$). One striking resemblance was it being withheld even after compensatory adjustment and this was consistent with a similar trend in the study conducted by other researchers (Lee *et al*., 2005).

5.1.2 Association between Magnesium and biochemical variables in the study population

The study provided information for the first time in Ghana on the serum magnesium level in cardiovascular disease persons and how an adjusted odds ratio with systolic blood pressure

(SBP) increments of ten (10) and BMI increment of five (5) could make magnesium levels closely relate with phosphate, calcium, creatine kinase (CK), diastolic blood pressure (DBP), albumin and total cholesterol. Finally, the hypotheses that; (1) there were no differences in serum magnesium and other biochemical factors and (2) that there were no differences between CVD cases and controls even after controlling for confounders were tested and found consistent with existing literature (Lee *et al.*, 2005 & Kieboom *et al.*, 2016), in that there were significant statistical differences. Results from the odds ratio showed that when confounders (e.g. SBP and BMI) were controlled and or adjusted, magnesium levels were associated with high density lipoprotein, albumin, phosphate, calcium and potassium levels.

In the present study, albumin, calcium, high density lipoprotein, phosphates and potassium influenced magnesium in CVD cases. Serum potassium levels were found to have a significant predictive value for hypomagnesaemia and this was consistent with other research work (Djagblatey *et al*., 2016). High serum potassium has been found elsewhere to protect against hypertensive and sodium-induced endothelial dysfunction independent of blood pressure (Sugimoto *et al.,* 1988; Volpe *et al.,* 1990). Intravenous potassium ameliorates hypertensive endothelial dysfunction in humans (Taddei *et al.,* 1994). This effect is lessened by N-monomethyl-L-arginine a competitive nitric oxide (NO) synthase inhibitor implicating the NO pathway. Potassium partly mediates vasodilation via strong inwardly rectifying potassium channels and the sodium-potassium-ATPase pump of vascular smooth muscle cells (Dawes *et al*., 2002). This might be important when NO bioavailability is low. Potassium blunts angiotensin-II–induced vasoconstriction (Novak and Harrison; 1973). Hence it was increasingly apparent that endothelial dysfunction was associated with a worse prognosis in cardiovascular disease (Suwaidi *et al*., 2000; Perticone *et al*., 2001). Potassium also has protective effects on

acute myocardiac infarction. Ischaemic myocardium extrudes potassium, causing hypopolarization and reducing the arrhythmic threshold (Harris *et al.,* 1954; Grumbach *et al.,*1954; Gettes *et al.,* 1966). Several large studies have shown an inverse relationship between blood pressure and potassium intake (Ascherio *et al.,* 1996; Geleijnse *et al.,* 1996). This antihypertensive effect may be mediated by increased natriuresis, vasodilation, heightened baroreflex sensitivity, and reduced cardiac sensitivity to catecholamines and angiotensin II (Barri & Wingo, 1997). However, concurrent repletion of magnesium with potassium with aldosterone blockade increases cellular potassium uptake and replenish tissue levels of both cations (French *et al.,* 1984). Potassium supplements and other potassium-sparing diuretics do not confer the same benefits (Farquharson & Struthers, 2002). High potassium intake reduces the risk of stroke (Khaw & Barret-Connor, 1987; Gillman *et al.,* 1995). However, potassium-rich diets tend to be low in sodium and high in anti-oxidants, fiber, and magnesium possibly confounds the issue.

The serum magnesium concentration as noted in this study, also, positively correlated with the serum concentration of other biochemical factors such as albumin, potassium, calcium, phosphorus and HDL in cardiovascular disease patients (Table 4.3) and these were consistent with de Baaij *et al.,* (2012). In their study, de Baaij *et al.,* (2012) observed atherogenic lipid profile characterized by increased total cholesterol, LDL cholesterol in newly diagnosed CVD. Low magnesium level and dyslipidemia observed in the current study might be predictive risk factors for CVD, thereby suggesting that magnesium supplementation, may prove to be beneficial to reduce the risk of CVD. A positive correlation between the serum magnesium and albumin may be expected in CVD, because the serum magnesium concentration includes complexed or protein-bound fractions (Ishimura *et al.,* 2007). Depending on the nature of

binding, there could possibly be spectator species that might lead to reduced states of these compounds. Hypoalbuminemia in CVDs has been attributed to a variety of factors, including exogenous albumin loss, albumin distribution, catabolism rate of proteins, and the presence of inflammatory cytokines (Don & Kaysen, 2000 & Cesari *et al.*, 2003). Serum albumin had been considered a surrogate marker of inflammatory status. However, controlling for inflammatory markers in a study alone by Williams *et al.,* (2008), had no effect on the association between serum albumin concentration and the incident of heart failure risk, suggesting that inflammatory status alone does not explain the albumin-heart failure link entirely (Williams *et al.*, 2008). Another alternative explanation was co-morbidities being associated with the development of heart failure that was associated with worsening serum albumin profile. Koch (2002), Huijgen *et. al* (2000), Chernow (1989) and their co-workers found no correlation between magnesium levels and serum albumin levels. However, in this study, inferential statistics revealed an association between magnesium and albumin which was consistent with other studies (Swaminathan, 2003). Hypomagnesemia, on the other hand, is being known to be frequently linked with hypokalemia (de Baaij *et al.*, 2012). Hypokalemia is induced when the rennin-aldosterone-angiotensin system and sympathetic nervous system are activated during a heart failure. High intake of diuretics also aggravates hypokalemia and heightens neuro-hormonal activation (Cleland *et al.,* 1987). Abnormal cellular ion transport resulting in altered membrane control over intracellular calcium might also be related to essential HHD. The free intracellular calcium concentration determines the tension in vessel smooth muscle cells, and thus peripheral vascular resistance (Chlumsky, 1993). In addition, studies have linked the fact that when there is excessive deposition in the vascular system it results in increased hardening of the arteries. This further leads to the elevation of the index of the mass of the left ventricle thereby contributing to the cause of carotid

vessel disease (Kendrick *et al*., 2010; Meng *et al*., 2010) with higher serum phosphorus concentrations.

5.2CONCLUSIONS

In conclusion, the cardiovascular disease patients and controls in this study were adult with majority having secondary school education and of Christian religious background. The study established statistically that there was a significant relationship between mean serum magnesium levels in the controls and cases in that magnesium level in the cases were lower in comparison to the controls. Despite this significance, further statistical analysis in accounting for confounding variables with or without rules established that there was a significant relationship between serum magnesium levels in the studied cases and controls. The study also established that serum magnesium level was positively associated with other biochemical parameters measured. The biochemical parameters such as serum potassium, phosphorous, HDL, LDL and albumin, FBG and CK levels were found to be significantly different between CVD patients and apparently healthy controls by being higher in the cases. The study in addition established a 13.33% increased risk of CVD prevalence in the participants with low magnesium levels. The study further showed that an adjusted odds ratio of 5.42 with systolic blood pressure increments of ten (10) and body mass index increment of five (5) could make magnesium levels closely relate with potassium, phosphate, calcium, CK, DBP, albumin and total cholesterol as determinants of risk of cardiovascular disease.

5.3 LIMITATIONS

The analysis measured total serum magnesium levels and not metabolically ionized magnesium as the methods required for the latter was not readily available. Although, the dry chemistry analytical method employed by the Vitrios 5.1 FS analyzer is used routinely in the hospital to determine serum concentration of magnesium, atomic absorption spectrophotometry method is the gold standard method in research for analysis of the magnesium. Serum troponins have been found in recent times to have excellent sensitivity and specificity and are superior to creatine kinase (CK-MB) as markers for myocardial dysfunctions but could not be included in the panels of biochemical analytes measured in the study due to its high financial cost of measurement. The effect of magnesium and the various biochemical parameters in the presentation of the various CVD cases sampled such as congestive heart failure, ischaemic heart disease, hypertensive heart disease and stroke should have been explored using statistically adequate numbers of each condition for comparison. Finally there were insufficient financial and material resources available for a larger sample size.

5.4 RECOMMENDATIONS

Considering that CVD has long term complications, it will be of significant interest to investigate extensively on magnesium and its contribution to the disease phenotype in CVD for point of care diagnostics. The gold standard of atomic absorption spectrophotometric method is to be used to see if similar trend can be established. Parathyroid hormone should be included in further work for further insights to the interplay among the electrolytes and the biochemical parameters. This study could be replicated with larger study population for stronger case for clinical policy formulation.

Since hormonal levels play crucial roles in biochemical parameters, analyzing hormonal levels in the current study might have been an added advantage. Also, the analysis measured total serum magnesium levels and not metabolically ionized magnesium as the methods required for the latter was not readily available. Although, this was the first report in the Ghanaian population, much work is needed in this regard. Also, considering that CVD has long term complications, it will be of significant interest to investigate extensively on magnesium and its contribution to the disease phenotype in CVD. When this is widely done, it would aid in point of care diagnostics for faster decision making in CVD management. The effect of magnesium and the various biochemical parameters in the presentation of the various CVD cases sampled should be explored in further studies using statistically adequate numbers of each condition for comparison.

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APPENDIX I

PARTICIPANT CONSENT INFORMATION SHEET

I am Michael Amewonye of the Department of Medical Laboratory Sciences, School of Biomedical and Allied Health Sciences (SBAHS), College of Health Sciences of University of Ghana. I am embarking on a study entitle ***Serum magnesium level and related biochemical parameters in cardiovascular diseases at the Korle-Bu Teaching Hospital***. The purpose of this study is to determine whether serum magnesium level is implicated in the pathogenesis of cardiovascular diseases (CVD). The development of CVD is due to many factors and one of it may be due to the inadequacy of magnesium level.

You will be asked few routine questions about your personal details and risk factors, etc. The benefits of the study, is to educate you on magnesium and its health benefits with respect to cardiovascular disease (CVD). It will be appreciated if you volunteer as a participant. Participation is entirely voluntary and strictly confidential .You may choose to withdraw from the study whenever you wish. Participants of the study will undergo an overnight fast after which 5mls of blood will be withdrawn. Both procedures may involve slight discomfort. The amount of blood to be taken by phlebotomists and used for this research study will not exceed 5mls.

You are assured of the strictest confidentiality of your personal information. This study will adhere to all applicable protocols and will maintain quality assurance in accordance with good laboratory practice. The blood samples collected will bear an identification code to ensure anonymity, confidentiality but afford ease of identification. There is the possibility that you might not benefit directly from participation. However, the information obtained and conclusions drawn will be applied in the adoption of relevant health policies as well as the appropriate care and management. You will incur no costs and you will also not be paid for participating in this study. However, you are entitled to know the outcome of the laboratory results and this will be well explained to you.

My mobile contact numbers is 0244664025. You may call me for any further clarification. Thank you for the cooperation and anticipated compliance to the study requirements including signing of this consent information form and the participatory consent form.

Signature/ Thump Print of Participant:…… Date: ……

Signature of Researcher:…….Date: ……

APPENDIX II

INFORMED CONSENT FORM

Research Title: **Serum magnesium level in cardiovascular disease and apparently healthy individuals in Korle-Bu Teaching Hospital**.

I, …… …….have been invited to take part in this research. I have been told of the purpose and procedure of this study which is to answer the questions raised about the effect of magnesium level on cardiovascular disease. I have agreed to give information about personal information such as my educational background, exposure to risk factors, etc. I understand that I will not be compensated monetarily for participating in this study. I am aware of the risk, dangers and discomforts that might be associated with the pain of blood collection. The study team will try to reduce the chances of those risks happening by employing trained health professionals including phlebotomists. The arm will be sanitized before blood collection, and new sterile needles and gloves will be used for each participant. I promise to comply with the requirement of study. I have therefore given my consent accordingly.

Signature/Thumbprint…….. Date…….

APPENDIX III

QUESTIONNAIRE

Participant's ID (Code) …….. Date……

A. SOCIO –DEMOGRAPHIC STATUS

1. Age ……. (years) 18-29 [ ] 30-39 [ ] 40-49[ ] 50 -59 [ ] 60 and above [ ]
2. Marital Status: Single [ ] Married [ ] Divorced [ ] Widowed [ ] Separated [ ]
3. Religion: Christian [ ] Muslim [ ] Others, please specify…….....
4. Education: No formal education [ ] Basic education (middle/JHS) [ ] SHS/O –Level [ ] HND/Diploma Certificate [ ] Bachelor Degree [ ] Postgraduate Degree [ ]
5. Employment Status: Employed [ ] Unemployed [ ] Retired [ ] Student [ ]
6. Are you a vegetarian? YES [ ] NO [ ]
7 If no, what is your typical regular meal? ……..
8. Do you have any medical condition? YES [ ] NO [ ]
9. If yes, please state the condition …….
10. Are you on any medication? YES [ ] NO [ ]

11b. If yes, mention the main drugs……

12a. Do you take other medication or herbal treatments? YES [ ] NO [ ]

12b. If yes, please state the name…….

B. LIFE STYLE BEHAVIOURS

13a. Do you smoke? YES [ ] NO [ ]

13b. If yes, how often? Daily [ ] Weekly [ ] Monthly [ ] Occasionally [ ]

14. Do you drink alcohol? YES [ ] NO [ ]

14b. If yes, how often? Daily [ ] Weekly [ ] Monthly [ ] Occasionally [ ]
One servings of alcohol: 120ml of wine (½ medium glass of dry wine)
285ml of beer (1/2 large beer bottle, one full mini Guinness)
30ml (1 tot of spirit, whisky gin, akpeteshi and alcoholic bitters)
60ml of (brandy, vermouth, aperitif)

14c. On average, how many servings of alcohol do you take daily? .....

15 Do you exercise regularly? YES [ ] NO [ ]. If so, which type of exercise? …… and how often do you exercise? ..... per week

16 Any other relevant comments
……
……

Thank you!

APPENDIX IV

DATA COLLECTION PROCEDURE

At the consent of a participant following adequate briefing;

1. Ask if a control participant has fasted for 8 to 12 hours and in case of a hospitalized subject, note the time of last meal and time of taking sample.

2. Check and record the blood pressure (BP) of participants in duplicate

3. If the systolic BP is within a range of 90 to 130mmHg and the diastolic BP is within the range 60 to 100mmHg, proceed to take weight and height measurement for the controls.

4. If a consented control subject is discovered to be hypertensive, discontinue with the procedure and direct him/her to seek treatment. This helps in avoiding waste of the questionnaire and minimizes errors.

5. However, if a CVD case is found to be hypertensive or not hypertensive, proceed to take weight and height measurement as in the case of the control subjects.

6. Please estimate weight and height figures or values from the folder records of stroke patients.

7. Administer the questionnaire, ensuring that each question is adequately filled in.

8. Proceed to take the following samples using a 5ml syringe and dispensing as follows:
   a) 3ml of blood into the coded gel separator tube or chemistry tubes provided and marching the code on the administered questionnaire.
   b) 1ml of blood into the same coded sodium fluoride tubes

9. Sort and transport the collected samples for processing, aliquoting and storage at -20^{o}C

10. Perform the following serum analysis on each sample: Mg^{2+}, Ca^{2+}, K^{+}, PO_4; Lipid profile; CK; FBG

11. Compile the results and submit to the Researcher/Investigator

APPENDIX V

OTHER DATA COLLECTION PROTOCOL

INCLUSION CRITERIA

Cases:

- ✓ All clinically confirmed **male** and **female** patients with congestive cardiac failure (CCF), Ischaemic heart disease (IHD), hypertensive heart disease (HHD), stroke (cardiovascular accident) and peripheral artery disease (PAD)

Controls:

- ✓ Apparently healthy male and female individuals without knowing or having previous record of hypertension, myocardial infarction or stroke, dyslipidaemia, diabetes, liver and kidney diseases or other metabolic syndromes.

Common inclusion criteria:

- All participants should be adults aging between 18 and 80 years
- Both case and control participants are recruited from KBTH
- All participants who have consented and signed the consent form.

EXCLUSION CRITERIA

All clinically confirmed male and female patients with congestive cardiac failure (CCF), ischaemic heart disease (IHD), hypertensive heart disease (HHD), stroke (cardiovascular accident) and peripheral artery disease (PAD).

Any subject that failed to meet the inclusion criteria above is excluded from participating in the study. Any participant unwilling to sign the consent form will be excluded from participating in the study.