THE DIAGNOSTIC UTILITY OF INFLAMMATORY MARKERS IN THE EVALUATION OF CARDIOVASCULAR RISK AMONG GHANAIANS PRESENTING WITH TYPE 2 DIABETES AND HYPERTENSION

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by

SYLVESTER YAO LOKPO

KWAME NKRUMAH UNIVERSITY OF SCIENCE & TECHNOLOGY,

KUMASI

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DECLARATION

I hereby declare that this submission is my own work towards the Masters and that, to the best of my knowledge, it contains no material previously published by another person, nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

Sylvester Yao Lokpo	……	……
(Student ID: PG6034911)	Signature	Date

Certified by:

Dr. W.K.B.A. Owiredu	……	……
Supervisor	Signature	Date

Certified by:

Prof. (Mrs) M.T. Frempong	……	……
Supervisor	Signature	Date

Prof. F.A. Yeboah	……	……
(Head of Department)	Signature	Date

ABSTRACT

It is suggested that inflammation is associated with hypertension and type 2 diabetes occurring either before or as a consequence of the development of these diseases. Hence inflammation measurement may provide additional information regarding a person's risk of cardiovascular morbidity and type 2 diabetes or contribute to the understanding of the pathogenesis of these diseases. This study sought to explore the utility of inflammatory markers in evaluating cardiovascular risk in Ghanaian type 2 diabetes and hypertensive subjects attending the Battor Catholic Hospital in the Volta Region. It also sought to determine the relative risk of developing cardiovascular disease among the different groups in the study population. This case-control study was conducted between December 2012 and February 2013, involving 125 participants comprising 43 hypertensives, 40 type 2 diabetics and 42 with both conditions. The control group consisted of 62 age-matched healthy individuals in the study area. Socio-demographic variables were obtained using a semi-structured questionnaire whiles other study parameters were obtained using standard methods. Inflammation was found to be associated with hypertensive and type 2 diabetic subjects recording higher levels of inflammatory markers (hs-CRP, IL-6, ESR and WBC) with the exception of TNF-α. Type 2 diabetes and hypertensive subjects also recorded significantly higher cardiometabolic risk profiles compared to their control peers. Twenty five percent (25%) of participants had a high risk of developing coronary disease over the next ten years. The percentage risk in general was higher in type 2 diabetes and hypertensive subjects compared to the healthy population. Gender variation in percentage ten year risk was observed with significant proportion of women recording high risk than men. Measurement of inflammation may be useful to identify cohorts of patients for clinical

trials to determine whether reduction/inhibition of hs-CRP reduces CHD events. Further studies could expand the scope of biomarkers of inflammation and endothelial dysfunction, compare oxidised LDL-Cholesterol levels between type 2 diabetes and hypertensive individuals with their healthy counterparts in evaluating their future cardiovascular risk. The sample size could also be increased substantially so that a generalised conclusion could be made among the Ghanaian population.

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DEDICATION

This thesis is dedicated to my family especially my father, Mr. George Kwaku Lokpo, my brother Festus Lokpo, my supervisors Dr. W.K.B.A Owiredu and Prof (Mrs) M.T. Frempong and Mr James Osei-Yeboah for his diverse contributions to the success of this work.

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 Glossary of terms

ACS: American Cancer Society

ACTH: Adrenal Corticotropic Hormone

AHA: American Hearrt Association

AIHW American Institute of Health and Welfare

ARIC: Atherosclerosis Risk In Communities

BMI: Body Mass Index

BP: Blood Pressure

CAD: Coronary Artery Disease

CATI-TRG: Computer Assisted Telephone Interviewing-Technical Reference Group

CCF: Congestive Cardiac Failure

CDC: Center for Disease Control

CHD: Coronary Heart Disease

CVA: Cardiovascular Accident

CVD: Cardiovascular Disease

DBP: Diastolic Blood Pressure

DECODA: Diabetes Epidemiology Collaborative Analysis of Diagnostics in Asia

DVT: Deep Vascular Thrombosis

EDTA: Ethylene diaminetetra acetic acid

ESR: Erythrocyte Sedimentation Rate

ESRD: End-Stage Renal Disease

FAO: Food and Agriculture Organisatioon

FH: Familial Hypocholesterlaemia

FHI: Family Health International

GH: Growth Hormone

GLUT4: Glutamine 4

GNA: Ghana News Agency

GPHC: Ghana Population and Housing Census

HC: Hip Circumference

HDL-C: High Density Lipoprotein-Cholesterol

HIV: Human Immunodeficiency Syndrome

hs-CRP: High-Sensitivity C-Reactive Protein

ICAM: Intercellular Adhesion Molecule

IDF: International Diabetes Federation

IFG: Impaired Fasting Glucose

IGT: Impaired Glucose Tolerance

IL-6: Interleukin-6

IR: Insulin Receptor

IRAS: Insulin Resistance Atherosclerosis Study

IRS-1: Insulin Receptor Substrate-1

ISH: International Society of Hypertension

KATH: Komfo Anokye Teaching Hospital

Kda: Kilo Daltons

KNUST: Kwame Nkrumah University of Science and Technology

LADA: Latent Autoimmune Diabetes in Adults

LDL-C: Low Density Lipoprotein- Cholesterol

LMIC: Low and Middle Income Country

MI: Myocardial Infarction

NCD: Non-Communicable Disease

NHLBI: National Heart Lung and Blood Institute

NO: Nitric Oxide

NPHP: National Public Health Partnership

OPD: Out Patient Department

Ox-LDL: Oxidised Low Density Lipoprotein

PAI-1: Plasminogen Activator Inhibitor-1

PE: Pulmonary Embolism

PHAC: Public Health Agency of Canada

PM: Particulate Matter

SBP: Systolic Blood Pressure

SMS: School of Medical Sciences

SSA: Sub-Saharan Africa

T2D: Type 2 Diabetes

TACE: TNF-Alpha Converting Enzyme

THI: Texas Heart Institute

TNF-A: Tumor Necrosis Factor- Alpha

TNFRI: Tumor Necrosis Factor Receptor I

VCAM: Vascular Adhesion Molecule

VLDL: Very Low Density Lipoprotein

WBC: White Blood Cell

WC: Waist Circumference

WHF: World Heart Foundation

WHO: World Health Organisation

WHR: Waist- to- Hip Ratio

CHAPTER ONE
INTRODUCTION

1.1 BACKGROUND

Cardiovascular disease (CVD) has become the number-one cause of death in the developing world (WHO, 2011). This epidemic has the potential to place a large social and economic burden on developing countries, where CVD tends to strike those in their prime working years (Gaziano, 2007).

The prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030 (Wild *et al.*, 2004).

Diabetes prevalence studies in southern Ghana have recorded a steady increase. Diabetes screening conducted by the Ghana Diabetes Association in the early 1990s suggested a 2–3% prevalence in urban areas in southern Ghana; in the late 1990s a prevalence rate of 6.4% for diabetes and 10.7% for impaired glucose tolerance (IGT) was recorded in a community in Accra (de-Graft Aikins, 2007).

There is accumulating evidence that inflammation is an important risk factor in CVD. It has been suggested that Type 2 diabetes may, in part, be precipitated or accelerated by an acute phase reaction as part of the innate immune response, in which large amounts of cytokines are released from adipose tissue, creating a lowgrade inflammatory milieu (Haffner, 2006). Therefore it is reasonable to imply that low-grade inflammation is an important pathogenesis factor in atherosclerosis

and cardiovascular events in patients with Type 2 diabetes (Haffner, 2006). Such systemic and subclinical inflammatory process can be characterized by elevated circulating levels of inflammatory cytokines including C-reactive protein (CRP) or high-sensitivity CRP (hs-CRP), interleukin-6 (IL-6), and tumour necrosis factor-alpha (TNF-alpha) (Lee and Liu, 2008).

1.2 STATEMENT OF THE PROBLEM

Cases of stroke were presented and treated at Korle-Bu hospital when it began operations in the 1920s. Between the 1920s and the 1960s data gathered from KorleBu hospital showed a steady increase of stroke and cardiovascular diseases. Hospital-based and community-based studies conducted since the 1950s provide important information on prevalence and morbidity trends for hypertension and diabetes, among others (de-Graft Aikins, 2007).

Globally, including Sub-Saharan Africa (SSA) consisting of those countries that are fully or partially located south of the Sahara Desert, certain risk factors have been found to account for up to 90%, of myocardial infarctions and other poor CVD outcomes such as stroke. These risk factors include smoking, alcohol consumption, obesity, diet, low physical activity, psychosocial factors, diabetes, hypertension and high lipid levels (BeLue *et al.*, 2009). Incidentally, these factors are also risk factors for the development of diabetes.

The surge in these traditional risk factors has largely been attributed to the fact that SSA is currently experiencing one of the most rapid epidemiological transitions characterized by increasing urbanization and changing lifestyle factors, which in

turn have raised the incidence of NCDs, especially CVD. Urbanization and economic development have also led to the emergence of a nutritional transition characterized by a shift to a higher caloric content diet and/or reduction of physical activity. Together, these transitions create enormous public health challenges, and failure to address the problem may impose a significant burden on the health sector and the economy of sub-Saharan African countries (BeLue *et al.*, 2009).

Some earlier publications have noted that traditional risk factors may not alone fully explain the excess risk conferred on persons with/or at risk of developing cardiovascular disease and type 2 diabetes and that the consideration of other "non-traditional" risk factors may be important to some extent, in fully explaining the underlying cause or pathogenesis of the disease in these individuals (Haffner *et al.*, 2002; Fonseca *et al.*, 2004). Currently as is the practice in Ghana, much premium is placed on the traditional risk factors such as obesity, elevated blood pressures, dyslipidaemia etc. in assessing future risk in most clinical settings by physicians.

1.3 JUSTIFICATION FOR STUDY

Over the last few years, there have been a lot of promising clinical markers proposed to link inflammation and atherosclerosis (Nystrom, 2007). Measuring inflammatory markers in serum may provide clinicians with additional information regarding a patient's risk of CVD (Kluft, 2004; Nystrom, 2007). Current evidence shows also that inflammatory cytokines play a role in the pathogenesis of diabetes and that inflammation measurement may increase diabetes risk prediction

(Lee and Liu, 2008).

On March 14 and 15, 2002, a workshop titled "CDC/AHA Workshop on Inflammatory Markers and Cardiovascular Disease: Applications to Clinical and Public Health Practice"sought to identify which inflammatory markers are suitable for use in clinical and public health settings in which it was unanimously recommended that hs-CRP among others be used **.** In spite of this recommendation there is paucity of evidence (data) to show that clinicians request laboratory investigation of these markers in assessing persons with cardiovascular risk(s) and type 2 diabetic clients who call at their facilities in Ghana.

Hence this study seeks not only to establish the association of these markers in clients visiting the Battor Catholic Hospital, in the North Tongu District of the Volta Region but the findings will seek to make a strong case for policy makers to include the assessment of at least one of these markers in the clinical evaluation of patients on a regular basis especially for those at high risk and also monitoring treatment response in persons on medication.

1.5 STUDY HYPOTHESIS

Inflammation is associated with hypertension and type 2 diabetes.

1.6 GENERAL OBJECTIVE

The aim of this research is to explore the diagnostic utility of inflammatory markers in the evaluation of cardiovascular risk in hypertension and type 2 diabetes.

1.6.1 Specifically

1. To measure inflammatory markers (hs-CRP, IL-6, TNF-α, ESR and WBC) associated with hypertension and type 2 diabetes.

2. To ascertain whether or not there are significant differences between the study participants and the healthy population with respect to the study variables.
3. To determine the relationship between inflammation and other cardiovascular risk factor variables among participants within the study population.
4. To determine the relative risk of developing Coronary Heart Disease (CHD) disease among the different groups within the general study population.

1.7 PROFILE OF STUDY AREA

The newly created North Tongu District, with its capital at Battor which retained the name of the old North Tongu – Adidome by Legislative Instrument (L1 2081) lies within latitudes $5^0$47'N to 6^0N and longitude $0^0$5' E to $0^0$45'E. It shares boundaries with Sogakope, and Ho Districts of the Volta Region: Asuogyaman District of the Eastern Region; Dangme-West and East Districts of the Greater Accra Region. The District's total land area of 1460 km^2 covers constututes 7.1% of the total land area of the Volta Region. The district was officially inaugurated on the 26th of June, 2012 (Republic of Ghana, 2013). The population of the District as recorded in the 2010 Ghana Population and Housing Census is 88, 388 with an estimated growth rate of 2.7% over the 2000 GPHC. Taking cognizance of the population figure and the growth rate, the District population is estimated at 96,613 as of 2012.

Figure 1.1: Poltical map of Ghana showing the Volta Region of

Ghana home to Battor, in the North Tongu District where the study was conducted. Source: UN (2005).

Figure 1.2: Poltical map of the Volta Region of Ghana home to Battor, in the North Tongu District where the study was conducted.

CHAPTER TWO

LITERATURE REVIEW

2.1 GLOBAL AND AFRICAN DIMENSIONS OF CARDIOVASCULAR DISEASE

Cardiovascular disease has become the number-one cause of death globally and in the developing world (WHO, 2011).This epidemic has the potential to place a large social and economic burden on developing countries, where CVD tends to strike those in their prime working years (Gaziano, 2007). In 2005, according to BeLue *et al.* (2009) an estimated 17.5 million people died of CVD, representing 30% of all global deaths. The overall burden continues to grow in both developed and developing countries. Nearly 80% of deaths in high-income countries occur among those over age sixty, compared with 42% in low and middle-income countries (Gaziano, 2007; BeLue *et al.*, 2009). Smith (2012) reported that, in 2004 an estimated 7.2 million deaths were due to coronary heart disease and 5.7 million were due to stroke. Over 80% of these deaths took place in Low and Middle Income Countries (LMICs) and occurred almost equally in men and women. By 2030, almost 23.6 million people will die from CVD; it is therefore projected to remain the single leading cause of death globally. Mortality from ischemic heart disease in these countries is expected to increase by 120% for women and 137% for men by 2020 (Smith, 2012). Four projections for the next 2 decades include an approximate tripling of ischemic heart disease and stroke mortality in Latin America, the Middle East, and sub-Saharan Africa (Kengne *et al.*, 2005) and relative to white subjects,

Afro-Caribbean and people of African descent have high incidence of stroke and end stage renal failure whereas coronary heart disease is less common (Ejim *et al.*, 2011).

2.2 BURDEN OF CARDIOVASCULAR DISEASE IN GHANA

In Ghana, several epidemiological studies have been conducted over the past 60 years. A survey conducted in a village about 60 miles from Accra in 1950 found that 5.5% of the 255 village inhabitants had cardiovascular diseases (Bosu, 2010). Nearly one quarter of the deaths in Mamprobi, Accra over the 1975-1980 periods was due to cardiovascular diseases and in 1981, the Ghana Health Assessment Team estimated that cerebrovascular disease and hypertensive heart disease accounted for 7% of the total healthy years-of-life lost (Bosu, 2010).

Henkle and Henkle (2012) noted that the West African country of Ghana has already seen a significant rise in the prevalence of hypertension, increasing in rural areas from 4.5% in 1977 to 24.1% in 2004. Over the same time period, there have been only seventeen population-based studies of hypertension conducted in Ghana. Of these studies, 8 surveyed rural populations and only one was conducted in the Volta Region.

The number of reported new cases of hypertension in outpatient public health facilities in Ghana increased more than ten-fold from 49,087 in 1988 to 505,180 in 2007. Over the same period, hypertension relative to the total reported outpatient

diseases increased from 1.7% to 4.0% in all ages (Bosu, 2010). In most regions, hypertension ranks as the fifth commonest cause of outpatient morbidity.

However, in the Greater Accra Region of Ghana, hypertension moved from fourth to become second to malaria as the leading cause of outpatient morbidity in 2007. Stroke and hypertension are among the leading causes of admission and death.

Hypertension is an important cause of heart and renal failure in Ghana (Bosu, 2010). In a recent report a prevalence rate of 36.4% Ghanaians in 2008 were hypertensive (WHO, 2011).

2.3 SOCIOECONOMIC BURDEN OF CARDIOVASCULAR DISEASE

The social impact has not been quantified to the same extent as the economic impact has been, but it includes loss of employment for the caregiver, relocation due to a loss of a job or the need to be closer to health care centers where they exist. Adolescents who become caregivers often drop out of the education system. The economic impact comes both in direct costs to the health care system and indirectly in losses to the economy through work loss both to those with disease and their family members who become de facto caregivers or who suffer loss of income because the bread-winner is not in the workforce (Gaziano, 2007).

A recent study of five countries emphasizes that a much higher proportion of deaths occur in the working age population in Brazil, India, and South Africa in contrast to the USA and Portugal. Despite limitations in regard to the quality of data collection, the potential consequences of the burden of CVD falling upon the

'breadwinners' of the community are sobering; Low and Middle Income Countries (LMIC) are faced with a dual burden of communicable and degenerative diseases which require tertiary care and a consequent diversion of limited resources. In conjunction with the loss of productive years of life, the consequences lead to economic constraints with an impact on both the private and the public sectors (Gersh, 2012).

In sub-Saharan Africa, the direct healthcare costs attributable to non-optimal blood pressure in 2001 was estimated at two billion US dollars. In the absence of adequate control measures, the prevalence of hypertension in some African countries has increased significantly to more than 30% (Bosu, 2010).

The actual financial burden of NCDs (CVD inclusive) on the budget in Ghana has not been quantified. However, the high expenditure involved in the management of the diseases cannot be underestimated. As the burden increases the risk factors worsen the cost of drugs, hospitalization and health infrastructure also increases (GNA, 2012). Indirect cost of loss of productivity sometimes exceeds direct cost. The chronic nature of NCDs makes them almost impossible for the patients to make optimal output at workplaces. Added to these, is the disproportionate effect on the poor, widening the economic gap as they spend substantial part of their incomes on drugs and the treatment while at the same time unable to generate enough income for their households due to ill-health (GNA, 2012).

TYPES OF CARDIOVASCULAR DISEASE

2.4.1Congenital heart disease

This is a heart defect present at birth. While some cases of congenital heart disease can be caused by genetic factors or by adverse exposures during pregnancy, the cause of most cases is unknown. Examples include holes between chambers of the heart (such as atrial septal defect or ventricular septal defect), abnormal valves, and abnormal heart chambers (Smith, 2012).

2.4.2 Stroke

A stroke occurs when the **blood** supply to the brain is interrupted. This can happen either when a blood vessel in the brain or neck is blocked or bursts. If this happens, the brain is deprived of oxygen and parts of the brain may be permanently damaged (WHF, 2012).

2.4.3 Inflammatory heart disease

This refers to inflammation of the heart muscle (myocarditis), the membrane sac which surrounds the heart (pericarditis), or the inner lining of the heart (endocarditis). Inflammation may be caused by known toxic or infectious agents or by an unknown origin (WHF, 2012).

2.4.4 Ischemic heart disease

It is the most common type of cardiovascular disease in Canada and other industrialized countries around the world. It refers to problems with the circulation of blood to the heart muscle. A partial blockage of one or more of the coronary arteries can result in a lack of enough oxygenated blood (ischemia) thus causing symptoms such as angina (chest pain) and dyspnoea (shortness of breath). A

complete blockage of an artery causes necrosis (damage to the tissues) or a myocardial infarction, commonly known as a heart attack (PHAC, 2010).

2.4.5 Coronary artery disease

In this type of disease, there is narrowing of the arteries supplying blood to the heart muscle due to a build-up of plaque (fatty deposits) that can lead to a heart attack or ischaemic heart disease (Smith, 2012).

2.4.6 Deep vein thrombosis and pulmonary embolism

The heart pumps oxygenated blood through the aorta to smaller arteries. After the blood supplies nutrients to vital organs, it returns through veins for reoxygenation in the lungs. Blood clots called deep vein thrombi (DVT) often develop in the deep leg veins. Pulmonary embolism (PE) occurs when clots break off from vein walls and travel through the heart to the pulmonary arteries. The broader term venous thromboembolism (VTE) refers to DVT, PE, or to a combination of both (Goldhaber and Morrison, 2002).

2.4.7 Rheumatic heart disease

Chronic condition resulting from preceding rheumatic fever (caused by streptococcal bacteria); damages the heart muscle and heart valves (Smith, 2012).

2.4.8 Risk factors associated with cardiovascular disease

The Australia Institute of Health and Welfare (AIHW) defines risk factors as determinants, characteristics or exposures that are associated with a greater risk of ill health. Risk factors are strongly influenced by variables such as a person's economic resources, working conditions, education, social support and access to

health care and social services. The specific risk factors for CVD, such as smoking and alcohol consumption, are strongly influenced by the wider circumstances in which people live and work. Importantly, the behavioural and physiological or clinical risk factors are critical as they can be modified, unlike heredity, sex and age (CATI-TRG/NPHP, 2003).

2.4.9 Physiological/Clinical risk factors

2.4.9.1 Abnormal blood lipids (fats):

Abnormal levels of lipids (fats) in the blood are risk factors for CVD. Cholesterol is carried through the blood by particles called lipoproteins: low-density lipoprotein (LDL) and high-density lipoprotein (HDL). High levels of LDL lead to atherosclerosis increasing the risk of heart attack and ischemic stroke. HDL reduces the risk of cardiovascular disease as it carries cholesterol away from the blood stream. High levels of triglyceride combined with high levels of LDL cholesterol speed up atherosclerosis thereby increasing the risk for heart attack and stroke (WHF, 2012).

2.4.9.2 Hypertension

The cause of hypertensive heart disease is chronically elevated blood pressure however; the causes of elevated blood pressure are diverse. Essential hypertension accounts for 90% of cases of hypertension in adults. Secondary causes of hypertension account for the remaining 10% of cases of chronically elevated BP. According to the Framingham Study, hypertension accounts for about one quarter

of heart failure cases. In the elderly population, as many as 68% of heart failure cases are attributed to hypertension. Uncontrolled and prolonged elevation of blood pressure can lead to a variety of changes in the myocardial structure, coronary vasculature, and conduction system of the heart (Riaz *et al.*, 2012).

2.4.9.3 Diabetic heart disease

People who have type 1 or type 2 diabetes can develop diabetic heart disease. The higher a person's blood sugar level is, the higher his or her risk of diabetic heart disease. Diabetes affects heart disease risk in three major ways. First, diabetes alone is a very serious risk factor for heart disease just like smoking, high blood pressure, and high blood cholesterol. In fact, people who have type 2 diabetes have the same risk of heart attack and dying from heart disease as people who already have had heart attacks. Second, when combined with other risk factors, diabetes further raises the risk of heart disease. Third, diabetes raises the risk of earlier and more severe heart problems (NHLBI, 2011).

2.4.10 Lifestyle (behavioural) risk factors

2.4.10.1 Obesity/overweight

The effects of obesity on cardiovascular health and disease are many, one of the most profound of which is hypertension. Risk estimates from population studies suggest that 75% of hypertension can be directly attributed to obesity. However, the precise mechanisms of hypertension related to obesity are not fully understood.

It is well documented that blood pressure increases with weight gain and decreases with weight loss (Krauss *et al.*, 1998).

Obesity has a strong effect on lipoprotein metabolism. Increased weight is a determinant of higher levels of triglycerides, elevated LDL-C, and low HDL-C. The association between obesity and LDL-C is more complex. LDL-C concentrations increase with BMI in men but BMI is associated with small, atherogenic LDL. Furthermore, central obesity in women is associated with elevated LDL-C concentrations. There is a strong link between obesity and a generalized metabolic disorder of which insulin resistance is an indicator (Krauss *et al.*, 1998).

2.4.10.2 Smoking

It has been well established that cigarette smoking is a powerful risk factor for coronary artery disease. A number of epidemiologic studies have shown a strong association between cigarette smoking and atherosclerosis, myocardial infarction and death from coronary artery disease. In addition to active smoking, passive smoking can also carry a risk of coronary artery disease. Although the detailed mechanism through which cigarette smoking is associated with cardiovascular disease has not yet been clarified, it is suggested that cigarette smoking is related to thrombogenesis, as well as atherogenesis, and blood platelet behavior is thought to be prominent among the proposed mechanisms involved in atherogenesis and thrombogenesis (Inoue, 2004).

2.4.10.3 Alcoholism

Diseases related to heavy consumption of alcohol and alcoholism include stroke, alcoholic cardiomyopathy, several kinds of cancer, cirrhosis, and pancreatitis, as well as accidents, suicide, and homicide. It is noted that heavy consumption of alcohol is a major cause of hypertension, so that the diseases related to hypertension, such as stroke, are generally related to alcohol consumption. Heavy consumption of alcohol also appears to affect heart muscle and possibly arterial tissues directly. Alcoholic cardiomyopathy is a common diagnosis in long-term alcoholics. While the relative and absolute risks of these diseases are negligible at one or two drinks per day, the mortality rates rise sharply (Pearson *et al.*, 2003) .

2.4.10.4 Physical inactivity

Sedentary lifestyles double the risk of cardiovascular diseases, diabetes, and obesity, and increase the risks of high blood pressure, lipid disorders, depression and anxiety. According to WHO, 60 to 85% of people in the world—from both developed and developing countries—lead sedentary lifestyles, making it one of the more serious yet insufficiently addressed public health problems of our time. It is estimated that nearly two-thirds of children are also insufficiently active, with serious implications for their future health (Seabra *et al.*, 2008).

2.4.10.5 Diet

A high intake of dietary fats strongly influences the risk of developing cardiovascular disease. Saturated fatty acids commonly found in dairy products

and meat raise cholesterol levels. Moreover, studies have also shown that transfatty acids, found in industrially hardened oils, increase the risk of coronary heart disease. While they have been eliminated from spreads in many parts of the world, trans fatty acids are still found in deep-fried fast foods and baked goods.

Cholesterol is not, in fact, required in the diet because it is produced by the liver in sufficient amounts. Dietary fibre is also a major factor in reducing total cholesterol in the blood and LDL cholesterol in particular. Eating a diet high in fibre and wholegrain cereals can reduce the risk of coronary heart disease. A high intake of salt (sodium) has been linked to high blood pressure, a major risk factor for stroke and coronary heart disease (WHO/FAO, 2003).

2.4.11 Demographic and hereditary risk factors

2.4.11.1 Age

The risk of heart disease increases about 3-fold with each advancing decade. Older age is considered a risk factor for heart disease after age 55 for women and after age 45 for men. This is partly because many women younger than 55 have not yet gone through menopause and still have high levels of the female hormone oestrogen in their blood. Oestrogen produced by the body is thought to help protect the heart. After menopause, however, the levels of oestrogen in a woman's body drop significantly. On average, women develop heart disease about 10 to 15 years later than men. Another reason that heart disease risk rises with age is because the build-up of fatty plaques in the arteries is a lifelong process. As you get older, blockages in the arteries get larger and may cause problems. These

blockages can reduce the amount of blood and oxygen that reaches the heart, causing chest pain or heart attack (Lansky *et al.*, 1998).

2.4.11.2 Gender/ Sex

There is a marked difference in coronary heart disease (CHD) risk between sexes. Among middle-aged people, CHD is 2 to 5 times more common in men than in women, and this sex ratio varies between populations. In both sexes, the risk of CHD increases markedly with age. The role of major cardiovascular risk factors in the development of CHD is well established among men. Among women, the data are less extensive. Reasons for the sex difference in CHD risk are not fully understood. Even though in most populations, cardiovascular risk factor patterns are more favourable among women than among men, very limited data are available to assess the extent to which cardiovascular risk factors can explain the observed sex difference in CHD risk (Jousilahti *et al.*, 1999).

2.4.11.3 Hereditary/Family history

Familial hypercholesterolemia (FH) is a common hereditary disease, characterized by elevated levels of plasma low-density lipoprotein cholesterol (LDL-C) and premature CVD. Characteristically, the mean age of onset of CVD is between 40 and 45 years in male FH patients and in female FH patients 10 years later. Nevertheless, the phenotypic expression of this disorder, in terms of onset and severity of atherosclerotic vascular disease, varies considerably (Jansen *et al.*, 2005).

2.4.12 Other risk factors

2.4.12.1 Stress

The stress of life have long been thought to increase a person's risk of cardiovascular disease or a serious coronary or cerebral event. In Australia; an expert group concluded that there is strong and consistent link between depression, social isolation and lack of quality social support and heart disease. These factors were as risky to health as abnormal blood lipid levels, smoking and high blood pressure. Elsewhere, other researchers have found a strong link between anxiety and heart disease. Studies show that acute stress triggers reduced blood flow to the heart, promotes your heart to beat irregularly and increases the likelihood of your blood clotting. All of these can trigger the development of cardiovascular disease (WHF, 2012).

2.4.12.2 Contraceptives

Early types of birth control pills contained high levels of oestrogen and progestin, and taking these pills increased the risk of heart disease and stroke, especially in women older than 35 years who smoked. If an individual smokes or have other risk factors, birth control pills will increase your risk of heart disease and blood clots, especially if you are older than 35 years. According to the American Heart Association, women who take birth control pills should have yearly check-ups that test blood pressure, triglyceride, and glucose levels (THI, 2012).

2.4.12.3 Air pollution

Evidence from epidemiological studies has consistently demonstrated that longterm exposure to ambient air pollutants is associated with increased cardiovascular morbidity and mortality. The American Cancer Society (ACS) cohort study reported that people living in more polluted areas with high levels of particulate matter (PM) with aerodynamic diameter <2.5 mm (PM2.5) were more likely to die from cardiopulmonary diseases and lung cancer than those in less polluted areas. An association between yearly average of particulate matter with aerodynamic diameter <10 mm (PM10) and increased risk for hospitalization for congestive heart failure or subsequent myocardial infarction has also been reported (Chuang *et al.*, 2010).

2.5 DIABETES-GLOBAL, AFRICA, SUB-SAHARAN AFRICA AND IN GHANA

The prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030; the total number projected to rise from 171 million in 2000 to 366 million in 2030 (Wild *et al.*, 2004). The urban population in developing countries is projected to double between 2000 and 2030. The most important demographic change to diabetes prevalence across the world appears to be the increase in the proportion of people greater than 65 years of age (Wild *et al.*, 2004).

At the beginning of the last century, diabetes mellitus was considered a rare medical condition in Africa, as illustrated by the famous statement of Dr Cook, who wrote ". . . diabetes is very uncommon but very fatal. . ." in his 1901 notes on the

diseases met in Africa (Kengne *et al.*, 2005). However, epidemiological studies carried out in the 90's have provided evidence of a different picture. The prevalence of type 2 diabetes is high among Africans Americans, Afro-Caribbean and among African migrants in Europe, all of them sharing genetic ancestry with black Africans. Diabetes and its long-term complications are higher in populations of African origin who have migrated to Western countries compared to Caucasians living in the same countries (Evaristo-neto *et al.*, 2010).

The estimated prevalence of diabetes in Africa is 1% in rural areas, up to 5% to 7% in urban sub-Saharan Africa, and between 8% and 13% in more developed areas such as South Africa and in populations of Indian origin. The annual incidence of type 1 diabetes mellitus varies from 4 to 10 per 100 000 among the 0- to 19-year-old population in Africa, with a high mortality rate. Although the majority of patients (70% to 90%) present with typical type 2 diabetes, up to 25% are considered to have type 1 diabetes. Among the latter group, it is currently estimated that approximately 15% may represent atypical presentations of diabetes, especially type 1B or ketosis-prone atypical diabetes, and tropical diabetes (Kengne *et al.*, 2005).

An aging population together with rapid urbanization will lead to an increase in the prevalence of diabetes such that by the year 2025, the majority of the world diabetes population will be living in the developing countries (Kengne *et al.*, 2005). Also, because of the adoption of Western lifestyles there is a global trend towards

an increased incidence and prevalence of diabetes mellitus in Africa. Indeed, Africa is experiencing one of the most rapid demographic and epidemiological transitions of the world history, characterized by a rise in the burden of non-communicable diseases (Evaristo-neto *et al.*, 2010).

In Sub-Saharan Africa in 2010, 12.1 million people were estimated to be living with diabetes in Africa, and this is projected to increase to 23.9 million by 2030. This trend is emerging in a region grappling with high rates of communicable diseases - including the highest global prevalence of HIV, Tuberculosis and Malaria. Diabetes is a component cause of several other important and often lethal diseases, both non-communicable diseases such as cardiovascular disease and renal disease, and communicable diseases such as pneumonia, bacteraemia and tuberculosis, which have considerable impacts on morbidity and mortality in the region (Hall *et al.*, 2011).

Diabetes prevalence studies in southern Ghana have recorded a steady increase. The earliest studies in the 1960s recorded 0.2% prevalence in a population of men in Ho (de-Graft Aikins, 2007). Diabetes screening conducted by the Ghana Diabetes Association in the early 1990s suggested a 2–3% prevalence in urban areas in southern Ghana; in the late 1990s a prevalence rate of 6.4% for diabetes and 10.7% for impaired glucose tolerance (IGT) was recorded in a community in Accra (deGraft Aikins, 2007). In 2014, there were about 450,000 cases of diabetes in Ghana;

the estimated prevalence among the adult population (20-79 years) stood at 3.3% (IDF, 2015).

2.5.1 Economic Impact of Diabetes

Economically, diabetes is an expensive disease, especially when the cost of complications, including the many diseases where diabetes is an underlying causal factor, is considered. The expenditure, as the International Diabetes Federation (IDF) has estimated in 2010 national funding for the healthcare of diabetics in Africa is just US$111 per person, which already amounts to 7% of national healthcare expenditure. With limited national funding, individual patients and their families may have to spend significant proportions of their income on treatment for diabetes, a level of expenditure that may not be sustainable or affordable (Hall *et al.,* 2011). The burden of type 2 diabetes is disproportionately borne by people of working age, which is also the age-group most profoundly affected by HIV in this region. Diabetes not only imposes considerable costs of treatment on families, it also hinders their ability to pay for this treatment through the loss of income of the diabetic member. At a national level an increasing prevalence of diabetes among the economically active, and the high prevalence of diabetic complications and low survival rates, will negatively impact economic development, and in turn the health budget (Hall *et al.,* 2011).

2.5.2 Risk factors associated with Type 2 Diabetes

There are marked differences between diabetic and non-diabetic individuals in the prevalence of some risk factors for diabetes and its complications, notably

anthropometric variables, such as obesity. Although it is true that these data are from cross-sectional studies that have limitations in establishing causality, they at least support the hypothesis that increasing prevalence of diabetes can be attributed largely to changes in lifestyle resulting in reduced physical activity and increased calorie intake and subsequent weight gain (Jamison *et al.*, 2006).

2.5.2.1 Obesity

Obesity and diabetes mellitus have a complex relationship, with type 2 diabetes strongly associated with obesity. Obesity stands out as a risk factor for type 2 diabetes, but we see some lean type 2 diabetes subjects probably having Latent Autoimmune Diabetes in Adults (LADA). Thus obesity may be a precursor for type 2 diabetes, following insulin resistance. Most researchers consider that this relationship is different in different types of obesity and type 2 diabetes. Further studies are needed to fully understand this. Causes of obesity are probably different for many types. Genetic disposition is clearly one. Different demographic groups according to lifestyle and genetics must be studied in a comprehensive way in order to understand more of these patterns (Hussain *et al.*, 2010).

2.5.2.2 Physical Activity

There seems to be a significant relationship between physical inactivity and diabetes and obesity. Physical activity is more common in rural than urban regions of Africa because rural populations rely on walking for transport and often have intense agricultural activities as their main occupation. In Sub-Saharan Africa,

walking time and pace is drastically reduced (by factors of 2 to 4 for walking at a slow pace and 6 to more than 10 for walking at a brisk pace) in an urban community as compared with a rural community. The main difference in physical activity between the two types of community, however, is the use of walking in rural areas as a means of transportation. The reduction in physical activity associated with life in a city partly explains the excess prevalence of obesity in urban areas. Thus, lack of physical activity appears to be a significant risk factor for diabetes in Sub-Saharan Africa (Jamison *et al.*, 2006).

2.5.2.3 Age and Ethnicity

Apart from the increasing prevalence rates in the Asian-Pacific region, the ages at which the disease develops are becoming younger. In developed countries with predominantly Caucasian populations, most people with diabetes are older than 65 years. In developing countries, however, the majority is between the ages of 45 and 64 years (The DECODA Study Group, 2003).

Ethnic differences in prevalence of type 2 diabetes are well documented. In 2004, the prevalence of diagnosed diabetes in the U.S. was higher for blacks and Hispanics than for whites across all age-groups. For women aged 45–64 years also, the prevalence was 7.8% among whites, 13.5% among Hispanics, and 15.4% among blacks. From 1980 through 2004, the age-adjusted prevalence increased by 65% among white women and 37% among black men (Shai *et al.*, 2006).

2.5.2.4 Urban-Rural Differences

Residence seems to be a major determinant of diabetes in Sub-Saharan Africa, since urban residents have 1.5- to 4.0 times higher prevalence of diabetes than their rural counterparts. This is attributable to lifestyle changes associated with urbanization and Westernization. Urban lifestyle in Africa is characterized by changes in dietary habits involving an increase in the consumption of refined sugars and saturated fat and a reduction in fibre. Some researchers have recently reported an increase in fasting plasma glucose in those whose lives have been spent in an urban environment, suggesting that both lifetime exposure to and recent migration to or current residence in an urban environment are potential risk factors for obesity and diabetes mellitus. The disease might represent the cumulative effects over years of dietary changes, decrease in physical activity, and psychological stress (Jamison *et al.*, 2006).

2.5.2.5 Family History

Probabilistically, a disease such as diabetes with a demonstrated genetic component is expected to cluster among relatives. Family history is a reflection of this fact with the added value that it also reflects the environment, cultural practices, and behaviours shared to some extent by close relatives. It has been amply documented that having one or more first-degree relatives with type 2 diabetes increases the odds of having the disease compared with someone without such relatives. The estimations vary, but the odds usually range from two to six times more likely. Also, a long-term study reported that the cumulative prevalence

of type 2 diabetes at age 80 years is about 3.5 times higher (38% vs. 11%) for people with a first-degree relative with type 2 diabetes compared to people without any affected relative (Valdez, 2009).

2.5.3 COMPLICATIONS OF DIABETES MELLITUS

2.5.3.1 Acute Complications

The three main metabolic complications of diabetes in Sub-Saharan Africa are diabetic ketoacidosis, hyperosmolar non-ketotic coma, and hypoglycaemia. Diabetic ketoacidosis is a common diabetic emergency in developing countries and carries with it relatively high mortality. The major contributing factors to such high mortality are the chronic lack of availability of insulin, delays in seeking medical assistance by newly diagnosed type 1 patients presenting in ketoacidosis, misdiagnosis of diabetes, and poor health care in general and diabetic care in particular. Hyperosmolar non-ketotic coma is usually a complication of type 2 diabetes and is less common and accounts for about 10 per cent of all hyperglycaemic emergencies in developing countries. Infection is the leading precipitating factor for both diabetic ketoacidosis and hyperosmolar non-ketotic coma, followed by first presentation of diabetes at a health institution and noncompliance with a medical regimen. Hypoglycaemia is also a serious complication of treatment in patients with diabetes (Jamison *et al.,* 2006).

2.5.3.2 Chronic Complications

The seriousness of diabetes is largely a result of its associated complications, which can be serious, disabling, and even fatal. Prevalence studies on complications

reported up to the early 1990s gave widely variable figures. These have been reviewed in two studies and include figures ranging from 9 to 16% for cataract, 7 to

52% for retinopathy, 6 to 47% for neuropathy, 6 to 30% for nephropathy, and 1 to 5% for macroangiopathy (Jamison *et al.*, 2006).

2.5.3.3 Cardiovascular Complications in Diabetes

Cardiovascular complications of diabetes, particularly the macrovascular varieties, are the result of chronic hyperglycaemia in association with classic and putative cardiovascular risk factors. Macrovascular complications tend to affect the heart (coronary artery disease), the central nervous system (cerebrovascular disease), and the lower limbs (peripheral vascular disease) (Kengne *et al.*, 2005).

Both type 1 and 2 diabetes are independent risk factors for CHD. Moreover, myocardial ischemia due to coronary atherosclerosis commonly occurs without symptoms in patients with diabetes. One reason for the poor prognosis in patients with both diabetes and ischemic heart disease seems to be an enhanced myocardial dysfunction leading to accelerated heart failure (diabetic cardiomyopathy). Thus, patients with diabetes are unusually prone to congestive heart failure (Grundy *et al.*, 1999a). From a study reported by Grundy *et al.* (1999a) , mortality from stroke was increased almost 3-fold when patients with diabetes are matched to those without diabetes. The most common site of cerebrovascular disease in patients with diabetes is occlusion of small paramedial penetrating arteries. Diabetes also

increases the likelihood of severe carotid atherosclerosis. Patients with diabetes, moreover, are likely to suffer irreversible brain damage with carotid emboli that otherwise would produce only transient ischemic attacks in persons without diabetes. Renal disease is a common and often severe complication of diabetes. When diabetes is present, CVD is the leading cause of death among patients with end-stage renal disease.

2.5.3.4 Inflammation and acute phase response

Inflammation is the local protective response to injury (C., 2004). It is a major and complex reaction of the body against infection upon tissue injury. It consists of recruitment and activation of leukocytes and plasma proteins at the site of infection to eliminate the infectious agent. The infectious microorganisms, after gaining bodily access to the site of injury, cause local inflammation. The local inflammatory response is later accompanied by a prominent systemic response known as acute phase response. This response is marked by the induction of fever, anorexia, solmonescence, lethargy, increased synthesis of hormones such as adrenocorticotropic hormone (ACTH) and hydrocortisone, increased leukocytosis and altered production of large number of proteins in liver. Those proteins whose levels change during inflammation are termed acute phase proteins (Khan *et al.*, 2012).

2.5.4 INFLAMMATION IN CARDIOVASCULAR DISEASE AND DIABETES MELLITUS

There is accumulating evidence that inflammation is an important risk factor in cardiovascular disease. Elevated levels of the inflammatory markers are associated with increased risk for CVD and diabetes mellitus (Haffner, 2006). It has also been suggested that type 2 diabetes may, in part, be precipitated or accelerated by an acute phase reaction as part of the innate immune response, in which large amounts of cytokines are released from adipose tissue, creating a low-grade inflammatory milieu (Nystrom, 2007). There is also firm evidence that atherosclerosis is an immune-mediated inflammatory disease. Therefore it is reasonable to imply that low-grade inflammation is an important pathogenesis factor in atherosclerosis and cardiovascular events in patients with type 2 diabetes (Nystrom, 2007). Such systemic and subclinical inflammatory processes can be characterized by elevated circulating levels of inflammatory cytokines including CRP or hs-CRP, IL-6 and TNF-α (Lee and Liu, 2008). TNF-α, IL-6 and CRP not only directly promote insulin resistance, but also stimulate endothelial production of adhesion molecules such as E-selectin, intercellular adhesion molecule-1 and vascular adhesion molecule-1, critical mediators of endothelial dysfunction in capillary and arteriolar endothelium. It has also been proposed that inflammatory cytokines secreted by adipose tissue exert an endocrine effect conferring insulin resistance in liver, skeletal muscle, and vascular endothelial tissue, ultimately leading to the clinical expression of both type 2 diabetes and cardiovascular disease (Hu *et al.*, 2004). Fibrinogen, which was previously recognized as an independent

coronary heart disease risk factor, is now considered an inflammatory marker and not only a coagulation component (Luc *et al.*, 2003).

Cross-sectional studies among non-diabetic subjects, individuals with impaired glucose tolerance (IGT)/impaired fasting glucose (IFG) or type 2 diabetic patients have confirmed that acute-phase reactants are positively correlated with measures of insulin resistance/plasma insulin concentration, BMI/waist circumference, and circulating triglyceride and negatively correlated with HDL cholesterol concentration. Others had found that after experimental induction of the acutephase response in illnesses in humans likely to be associated with an acute-phase response there are elevated serum concentrations of total cholesterol and VLDL triglyceride and lowered HDL cholesterol-typical features ("dyslipidemia") of type 2 diabetes (Pickup, 2004).

Reports of De Rooij *et al.* (2009) showed that white blood cell count (WBC) was related to elements of the metabolic syndrome in the Insulin Resistance Atherosclerosis Study (IRAS) among a non-diabetic population. The Atherosclerosis Risk in Communities (ARIC) study also recorded association between raised WBC and diagnosis of diabetes seven years later in a large middleaged population. Similarly, in a large cohort study in patients undergoing angiography, the erythrocyte sedimentation rate (ESR) was related to coronary atherosclerosis and was a predictor of cardiac death in patients with probable ischemic heart disease.

2.5.4.1 C-reactive protein, Interleukin-6, Tumor necrosis factor-alpha, their molecular structure and function

2.5.4.1.1 C-reactive protein (CRP)

CRP, is so named for its capacity to precipitate the somatic C-polysaccharide of Streptococcus pneumoniae, and was the first acute-phase protein to be described (Pepys and Hirschfield, 2003). IL-6 is the major initiator of acute phase response by hepatocytes and a primary determinant of hepatic CRP production (Luc *et al.*, 2003). It is a normal plasma protein, the circulating concentration of which rises dramatically in response to most forms of tissue injury, infection and inflammation, and serum values are widely measured in clinical practice as an objective index of disease activity (Thompson *et al.*, 1999).

Structurally, the human CRP molecule is composed of five identical nonglycosylated polypeptide subunits each containing 206 amino acid residues. The protomers are non-covalently associated in an annular configuration with cyclic pentameric symmetry. The CRP protomer has the characteristic 'lectinfold' composed of a two layered sheet with flattened jellyroll topology. Two calcium ions are bound 4A° apart by protein side-chains coming from loops at the concave face, termed the B face as this is the site of ligand binding. The other face (A) carries a single a helix (Figure 1).

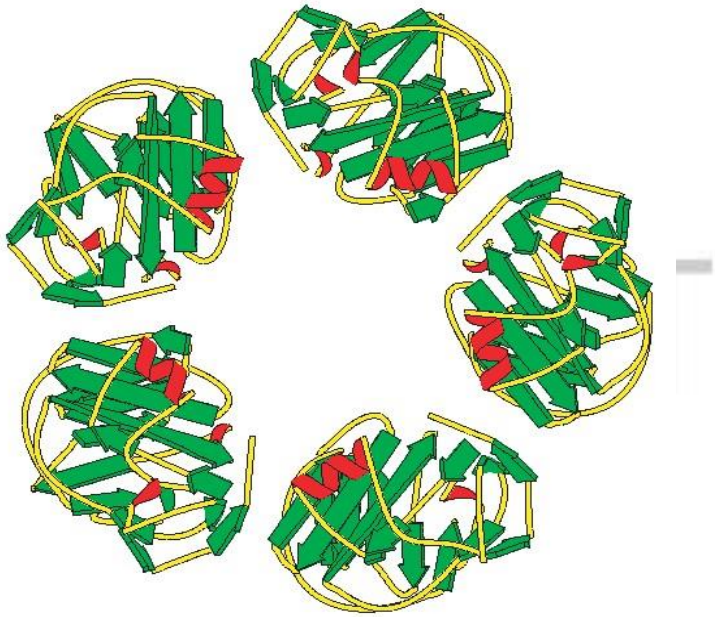

Figure 2.1: Molecular structure of CRP, viewed face-on showing the A face, displayed as a ribbon diagram

The crystallographic structure of the CRP-PC complex, and indirect evidence from mutagenesis studies, show that the key residues in the ligand binding pocket responsible for recognition of PC are Phe66 and Glu88. C1q, the recognition protein of the classical pathway, probably binds to complexed or aggregated CRP in a pocket at the open end of a cleft on the A faces of the protomers in the intact pentamer. Mutagenesis studies of CRP suggest that Asp112 and Tyr175 are important contact residues for C1q binding that Glu88 influences the conformational change in C1q necessary for complement activation, and that Asn158 and His38 probably contribute to the correct geometry of the binding site (Hirschfield *et al,* 2003). Functionally, human CRP binds with highest affinity to phosphocholine residues, but it also binds to a variety of other autologous and

extrinsic ligands, and it aggregates or precipitates the cellular, particulate, or molecular structures bearing these ligands. Autologous ligands include native and modified plasma lipoproteins, damaged cell membranes, a number of different phospholipids and related compounds, small nuclear ribonucleoprotein particles, and apoptotic cells. Extrinsic ligands include many glycan, phospholipid, and other constituents of microorganisms, such as capsular and somatic components of bacteria, fungi, and parasites, as well as plant products. When aggregated or bound to macromolecular ligands, human CRP is recognized by C1q and potently activates the classical complement pathway, engaging C3, the main adhesion molecule of the complement system, and the terminal membrane attack complex, C5–C9. Bound CRP may also provide secondary binding sites for factor H and thereby regulate alternative-pathway amplification and C5 convertases (Pepys *et al*, 2003). The secondary effects of CRP that follow ligand binding resemble some of the key properties of antibodies, suggesting that under various circumstances CRP may contribute to host defense against infection, function as a pro-inflammatory mediator and participate in physiological and pathophysiological handling of autologous constituents (Hirschfield and Pepys, 2003).

2.5.4.1.2 Interleukin-6 (IL-6)

IL-6 is a cytokine characterized by its pleiotropic action (Toumpanakis and Vassilakopoulos, 2007). It is involved in a myriad of biologic processes, perhaps explaining its long list of synonyms (B-cell stimulatory factor-2, B cell differentiation factor, T cell-replacing factor, interferon-b2, 26-kDa protein,

hybridoma growth factor, interleukin hybridoma plasmacytoma factor 1, plasmacytoma growth factor, hepatocyte-stimulating factor, macrophage granulocyte-inducing factor 2, cytotoxic T cell differentiation factor, thrombopoietin) (Keller *et al.*, 1996). IL-6 is synthesized by many different cells. The major IL-6-producing cells are monocytes/macrophages, endothelial cells and fibroblasts (Schooltink *et al.*, 1991) and IL-6 expression is regulated by a variety of factors, including steroidal hormones, at both the transcriptional and posttranscriptional levels. IL-6 achieves its effects through the ligand-specific IL-6 receptor (IL-6R) (Keller *et al.*, 1996).

Human IL-6 is a protein with a molecular weight of 21kDa-28kDa (Toumpanakis and Vassilakopoulos, 2007) depending on post-translational processing such as glycosylation and phosphorylation. The IL-6 peptide contains 212 amino acids of which a 28 amino acids hydrophobic signal peptide is cleaved off resulting in a mature protein of 184 amino acids. The human IL-6 gene, located on chromosome 7, is approximately 5 Kb (compared to 7 Kb for the mouse) and consists of four introns and five exons. The human IL-6 gene contains three transcriptional initiation sites which correspond with three TATA-like sequences (Keller *et al.*, 1996).

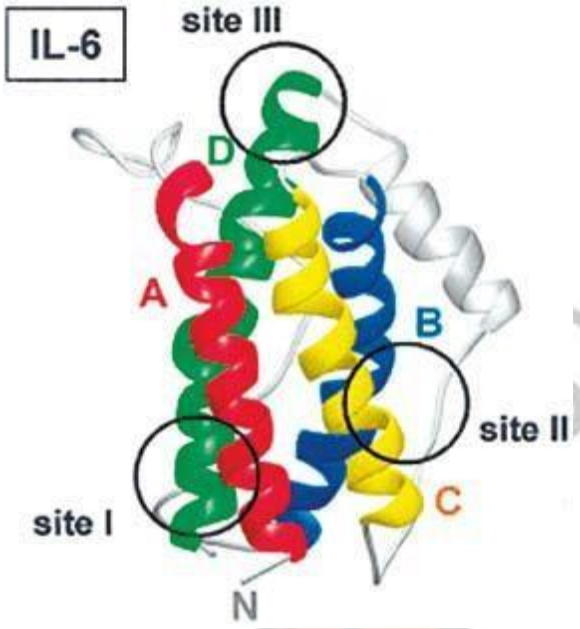

Figure 2.2: Tertiary Structure of IL-6. IL-6 is composed of four a-helices (coloured) linked via connecting loops (grey). The figure also shows IL-6 receptor-binding sites, named sites I, II and III.

Crystallography X showed that IL-6 is formed by 4 a-helices, arranged as two couples of anti-parallel helices (Figure 2), a common mode in the cytokine family. According to the length of the a-helices, IL-6 is part of the "long-chain" cytokine family, which also includes growth hormone (GH), erythropoietin and G-CSF factor. Based on mutagenesis studies, three sites on the IL-6 molecule have been recognized to mediate IL-6 binding to its receptors. IL-6 binds to two different membrane glycoproteins (receptors) that together form the common IL-6 receptor. These receptors are type I membrane proteins, i.e. they contain a transmembrane domain and an extracellular N-terminal domain. Three domains of IL-6 have been recognized that mediate IL-6 binding to its receptors, namely sites I, II and III (Toumpanakis and Vassilakopoulos, 2007). Functionally, IL-6 is now well

recognized for its role in the acute phase inflammatory response which is characterized by production of a variety of hepatic proteins termed acute phase proteins. It is important for the development of specific immunologic responses. IL-6 induces differentiation of activated, B cells culminating in production of immunoglobulin. Along with B cell differentiation, it stimulates proliferation of thymic and peripheral T cells and in cooperation with IL-1, induces T cell differentiation to cytolytic-T cells and activates natural killer cells. IL-6 also appears to play an important role in bone metabolism through induction of osteoclastogenesis and osteoclast activity. It functions in a wide variety of other systems including the reproductive system by participating in menses and spermatogenesis, skin proliferation, megakaryocytopoiesis, macrophage differentiation, and neural cell differentiation and proliferation (Keller *et al.*, 1996).

2.5.4.1.3 Tumor necrosis factor-alpha (TNF-alpha)

TNF-alpha is a potent pro-inflammatory cytokine exerting pleiotropic effects on various cell types and plays a critical role in the pathogenesis of chronic inflammatory diseases (Horiuchi *et al.*, 2010). It is produced by many different cell types. The main sources in vivo are stimulated monocytes, fibroblasts, and endothelial cells. Macrophages, T-cells, B-lymphocytes, granulocytes, smooth muscle cells, chondrocytes, osteoblasts, mast cells, glial cells, and keratinocytes also produce TNF-α after stimulation. Glioblastoma cells constitutively produce TNF-α and the factor can be detected also in the cerebrospinal fluid. Human milk also contains TNF-α (Mukhopadhyay *et al.*, 2006). TNF-α is generated as a precursor

form called transmembrane TNF-α that is expressed as a cell surface type II polypeptide consisting of 233 amino acid residues (26 kDa) on activated macrophages and lymphocytes as well as other cell types. After being processed by such metalloproteinases as TNF-alpha-converting enzyme (TACE) between residues alanine76 and valine77, the soluble form of TNF-alpha of 157 amino acid residues (17 kDa) is released. Soluble TNF-α is a homotrimer of 17-kDa cleaved monomers and transmembrane TNF-α also exists as a homotrimer of 26-kDa uncleaved monomers. Transmembrane TNF-α is palmitoylated at a specific cysteine residue located just at the boundary between the transmembrane and the cytoplasmic domains. In addition, serine residues of the intracellular domain of transmembrane TNF-α are phosphorylated. These kinds of post-translational modification may be important for the regulation of transmembrane TNF-alpha function. After releasing soluble TNF-α by TACE cleavage, the residual cytoplasmic domain of transmembrane TNF-α migrated back into the nucleus of the transmembrane TNF-alpha-bearing cells (Horiuchi *et al*., 2010).

To date most (if not all) of the cellular actions of TNF-α have been attributed to the activities of two distinct receptors: type 1 (TNFR1, a 55- or 60-kDa peptide in rodents and humans), respectively; and a type 2 (TNFR2, a 75- or 80-kDa in rodents and humans), respectively. Both of these receptors are expressed ubiquitously

(albeit at different ratios) and oligomerise upon ligand binding (Sethi and Hotamisligil, 1999). TNF-α is a multi-functional cytokine that can regulate many

cellular and biological processes such as immune function, cell differentiation, proliferation, apoptosis and energy metabolism. TNF-α affects glucose homeostasis in adipocytes, promotes lipolysis in cultured adipocytes and potently inhibits adipocyte differentiation and lipogenesis. TNF-α suppresses the expression of many proteins that are required for insulin-stimulated glucose uptake in adipocytes, such as the insulin receptor (IR), insulin receptor substrate-1 (IRS-1) and GLUT4 (Cawthorn and Sethi, 2008).

2.5.5 ROLE OF INFLAMMATORY MARKERS IN THE MECHANISM OF ATHEROSCLEROTIC PROCESS

Atherosclerosis is the underlying cause of most CVD, starting early in life and progressing slowly and silently for decades before being complicated. The development of atherosclerosis involves a complex and self-reinforcing interaction between lipid accumulation and modification, the endothelium, smooth muscle cells and macrophages, inflammatory cytokines and various blood elements (Kluft, 2004). It is therefore the descriptive term for thickened and hardened lesions of the medium and large muscular and elastic arteries. These lesions are lipid rich and occur within the intima, although the media and adventitia may also be involved. Lesions or plagues are generally eccentric and, if they become complicated by mural or occlusive thrombosis, may cause ischemia with onset of clinical angina or necrosis with the characteristic clinical sequelae of myocardial infarction (MI), cerebral infarction, or gangrene of the extremities; hence, the term atherothrombosis (Goldman and Ausiello, 2007).

Endothelial injury has been proposed to be an early and clinically relevant pathophysiologic event in the atherosclerotic process (González-Gay and González-Juanatey, 2012) manifesting as deficiencies of nitric oxide (NO) and prostacyclin. This can be induced by various noxious insults including dyslipidemia, diabetes, hypertension, smoking, etc. (Fonseca *et al.*, 2004). Recent data suggest that the prediabetic state may be associated with endothelial dysfunction possibly due to Insulin Resistance. The next event in atherogenesis is the binding of mononuclear cells, such as monocytes and T lymphocytes, to the endothelium; this binding is mediated by adhesion molecules present on the endothelial surface, such as vascular cell adhesion molecule (VCAM), intercellular adhesion molecule (ICAM), and E-selectin. Once the monocyte migrates into the subendothelial space, it matures into a resident macrophage, takes up lipid largely through certain scavenger receptors such as SR-A and CD-36, and becomes a foam cell. In the later stages of atherogenesis, smooth muscle cells migrate to the surface and form the fibrous cap of the lesion. Finally, lipid-laden macrophages release matrix metallo-proteinases causing plaque rupture and acute coronary syndromes such as myocardial infarction and unstable angina (Fonseca *et al.*, 2004).

Oxidative stress plays a crucial role in atherogenesis, especially in diabetes. Several lines of evidence support a pro-atherogenic role for oxidized low-density lipoprotein (Ox-LDL) and its in vivo existence. Ox-LDL is not recognized by the LDL receptor but by the scavenger receptor pathway on macrophages, which results in unregulated cholesterol accumulation, leading to foam cell formation.

Factors that may promote increased oxidative stress in diabetes include antioxidant deficiencies, increased production of reactive oxygen species, and the process of glycation and glycol-oxidation (Fonseca *et al.*, 2004).

2.5.6 THE ROLE OF INFLAMMATORY MARKERS IN THE DEVELOPMENT OF INSULIN RESISTANCE AND TYPE 2 DIABETES.

Various mechanisms have been indicated by which cytokines can contribute to the development of insulin resistance and type 2 diabetes. For example, cytokines can directly inhibit insulin receptor signaling by activating c-Jun amino-terminal kinase and an inhibitor of nuclear factor kappa-beta kinase, leading to serine phosphorylation of insulin receptor substrate-1(Lee and Liu, 2008). Potential indirect mechanisms linking inflammatory parameters to insulin resistance are more plentiful. For example, TNF-α, IL-6 and IL-1 can promote adipocyte lipolysis and de novo hepatic fatty acid synthesis – a consequent increase in fatty acids, may lead to impaired hepatic and peripheral insulin metabolism and function (Sattar *et al.*, 2003). An alternative explanation proposes endothelial dysfunction as an intermediate between inflammation and insulin resistance. The endothelium plays an important role in regulating blood flow and thus glucose uptake in insulin sensitive tissues. Although the physiological importance of the latter mechanism remains controversial, circulating factors that impede endothelial-dependent vasodilatation may reduce glucose uptake in response to insulin. Acute elevations in circulating cytokines impair endothelial function and this phenomenon appears to hold true for chronic low-grade inflammation. Interestingly, low whole blood

production capacity of the anti-inflammatory cytokine IL-10 (i.e. a proinflammatory response) may enhance risk of diabetes development by endothelial dysfunction: IL-10 appears to preserve endothelial function during acute inflammation by reducing superoxide generation (Sattar *et al.*, 2003). Finally, and of greatest current interest is the observation that adiponectin, an adipose tissuespecific plasma protein with potent anti-inflammatory properties, circulates at lower concentrations in obese individuals, increases with weight loss, and most importantly predicts changes in insulin sensitivity and development of diabetes (Sattar *et al.*, 2003).

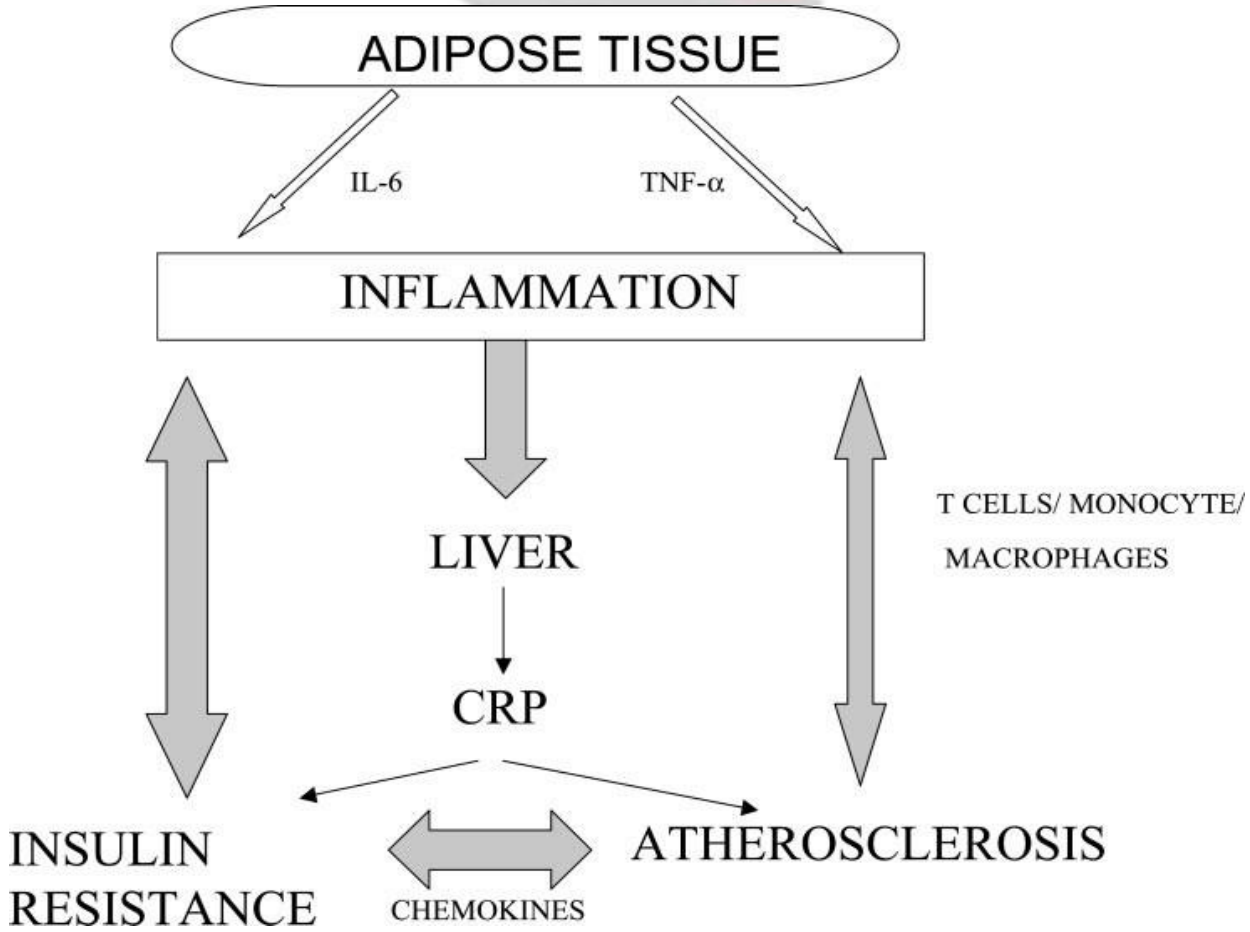

Figure 2.3 Schematic representation of the interactions between inflammation, IR, and atherosclerosis

CHAPTER THREE

MATERIALS AND METHODS

3.1 STUDY DESIGN

The study was a prospective case-control

3.2 STUDY SETTING

The study was conducted from December 2012 to February 2013 at the Battor Catholic Hospital, in the North Tongu District of the Volta Region of Ghana.

3.3 SAMPLING TECHNIQUES, SAMPLE SIZE

A total of 187 study subjects were selected into the study. These consisted of 125 case and 62 control subjects. The case subjects were defined as persons with hypertension and/or type 2 diabetes. However the control subjects included persons without hypertension and diabetes and were mainly blood donors, patient relatives and staff who consented to be part of the study. The case subjects were stratified into the following three groups; 43 known hypertensives, 42 known hypertensives and diabetics and 40 known diabetics only. The subjects comprised clients who visited the Battor Catholic Hospital and were seen at the Out-Patient Department (OPD).

3.3.1 Known hypertensive group

The selection of the 43 participants into this group was based on the following criteria;

1. previously diagnosed hypertension
2. on anti-hypertensive medication

However no distinction was made between primary and secondary hypertension.

3.3.2 Known hypertensive and type 2 diabetic group

The 42 hypertensive and diabetic group were categorized based on these criteria;

1. previously diagnosed of both hypertension and type 2 diabetes
2. on medications for both hypertension and type 2 diabetes

3.3.3 Known type 2 diabetic group

The 24 participants were selected based on the following;

1. diagnosed previously of type 2 diabetes
2. on diabetic medication

3.3.4 Control group

Controls included male and female with no histories of hypertension and diabetes. 62 participants were selected into this group.

The diagnosis of hypertension and diabetes were done using the following;

1. The WHO/IDF diagnostic criteria for diabetes – fasting plasma glucose ≥ 7.0mmol/l (126mg/dl) or 2–h plasma glucose ≥ 11.1mmol/l (200mg/dl)
2. The WHO/ISH diagnostic criteria for hypertension- systolic pressure ≥140mmhg and diastolic pressure ≥ 90mmhg

3.4 INCLUSION CRITERIA

1. Males and females 20 years and above.
2. Newly diagnosed and known type 2 diabetics
3. Known hypertensives

4. Non pregnant women

5. Controls included males and females without any hypertension and diabetes

3.5 EXCLUSION CRITERIA

1. Males and females less than 20years
2. Type 1 diabetics.
3. Client with existing cardiovascular disease (CVA, CCF, CAD etc.)
4. Client with any form of infection (septicaemia, hepatitis, dermatitis, candidiasis etc.)
5. Client with any form of inflammatory disease (asthma, rheumatoid arthritis, pelvic inflammatory disease, cancer etc.)
6. Pregnant women
7. Just undergone surgery
8. Clients on lipid lowering drugs (e.g. statins, fibrates, niacinetc)
9. Heavy smokers (more than one pack per day)
10. Clients with medical and out patients 'records not available for review.

LABORATORY TESTS AND OTHER MEASUREMENTS

3.6.1 Blood pressure measurement

Mercury sphygmomanometer and stethoscope were used to measure the blood pressures after at least a 10 minute rest in according to the recommendation of the American Heart Association (Kirkendall *et al.*, 1967). In ensuring accurate readings, an appropriate-50 size blood pressure cuff was used and blood pressures of each patient were taken twice by a single qualified nurse within an interval of 5 minutes and the average value taken.

3.6.2 Measurement of Anthropometric indices

The anthropometric measurements were made using the method described by Bannerman *et al.* (2002). Measurements of height (to the nearest 0.1 cm) were made using a portable standiometer (height meter) and body weight (to the nearest 0.1 kg) using a portable weighing scale. Subjects were measured in light clothing without shoes. Then BMI was calculated by the formula: BMI = weight (kg)/height^2 (m)^2. The waist circumference was measured at the point yielding the smallest circumference between the lower rib margin and the iliac crest. Hip circumference was recorded at the point yielding the maximum circumference over the buttocks. The waist-to-hip ratio (WHR) was then calculated.

3.6.3 Sample collection and preparation

About 7ml of fasting venous blood was drawn from each subject fasting overnight (12-14 hours) using standard procedures. 2ml of whole blood was taken into EDTA.K3 sterile vacuum tube and mixed well to ensure homogenous mixture. Another 2 ml was discharged into BD vacutainer® serum separator tubes for the estimation of different biochemical parameters. 0.8 ml of the blood was dispensed into a prefilled vial (0.2 ml of 3.8% sodium citrate used as diluents making a 4:1 dilution). 2ml of the rest was then put into sodium fluoride tube and mixed adequately. The venous blood was drawn with the patient in a sitting position. Venipuncture was done using a tourniquet and minimum stasis was also employed to avoid elevation in the biochemical indices.

The samples were immediately transported to the clinical biochemistry and haematology laboratories of Battor Catholic Hospital, where clotted blood was centrifuged at 3000g for 5 minutes to obtain serum and the serum was stored at -20 °C until analysis. Biochemical indices such as hs-CRP were measured using i-CHROMA™ reader system (Boditech Med Inc. Chuncheon, Korea), lipid profile were assayed using BT 3000® (Biotenica Instruments, Italy) a clinical chemistry auto analyser. Serum for IL-6 and TNF-α were measured on Tecan Absorbance Microplate Reader (Sunrise) system (Tecan Trading AG, Switzerland). EDTA.K3 blood for the estimation of haemoglobin level and total white blood cell count was done using Sysmex KX-21N (Sysmex Corporation, Japan) automated haematology analyser. The auto analysers were calibrated before the start of the analysis. Results flagged as high or low were repeated to verify their reproducibility. Sample for fasting blood glucose was estimated using a semi-auto biochemistry analyser (Maysun Company Limited, China) and the citrated whole blood sample was used for the estimation of ESR using Westergren ESR Kits (Guest Scientific Swaziland).

3.6.4 Analysis of blood glucose and other biochemical variables

Plasma sample was used to estimate fasting blood glucose using ELITech reagents from Vital Scientific Co Ltd. Stored frozen serum was thawed adequately at room temperature and the aliquots were used to measure i-CHROMA™ hs-CRP using kits from Boditech Med Inc. Chuncheon (Korea), IL-6 and TNF-a levels analysed using MABTECH AB

Sweden (2012 and 2013) reagents. Total Cholesterol, HDL-Cholesterol and Triglyceride were also assayed using reagents from JAS™ Diagnostics, Florida. LDL-Cholesterol and VLDL were however calculated using the Friedwald *et al.* (1972) and DeLong *et al.* (1986) equations respectively.

3.6.4.1 Glucose Determination

Principle and Methodology

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

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

$$2H_2O_2 + \text{Phenol} + \text{4-aminoantipyrine} \xrightarrow{\text{peroxidase}} \text{Quinoneimine} + 4H_2O$$

3.6.4.2 Cholesterol Determination

Principle and Methodology

The method uses Trinder (1969) colour system of peroxidase / phenol/ 4 aminoantipyrine. Cholesterol esterase hydrolyses esters to free cholesterol and fatty acids. The free cholesterol produced plus the preformed cholesterol are then oxidized in the presence of cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide. The quinoneimine chromogen, with absorption maximum at 540-550 nm, is produced when phenol

substitute (HBA) is oxidatively coupled with 4-aminophenazone in the presence of peroxidase with hydrogen peroxide.

Cholesterol ester + H_2O $\xrightarrow{\text{cholesterol esterase}}$ Cholesterol + Fatty acids

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

$2H_2O_2$ + HBA + 4-aminoantipyrine $\xrightarrow{\text{peroxidase}}$ Quinoneimine + $4H_2O$

The intensity of the final red colour is directly proportional to the total cholesterol concentration.

3.6.4.3 HDL-Cholesterol Determination

Principle and Methodology

The assay is based on a modified polyvinyl sulfonic acid (PVS) and polyethylene-glycolmethyl ether (PEGME) coupled classic precipitation method with the improvements in using optimized quantities of PVS/PEGME and selected detergents(). LDL, VLDL and chylomicrons (CM) react with PVS and PEGME and the reaction results in accessibility of LDL, VLDL and CM by cholesterol oxidase and cholesterol esterase. The enzymes selectively react with HDL to produce H_2O_2 which is detected through a Trinder reaction.

HDL + LDL + VLDL + CM $\xrightarrow{\text{PVS/PEGME}}$ HDL + (LDL + VLDL + CM). PVS/PEGME

HDL + Cholesterol esterase + Cholesterol oxidase $\rightarrow$ Fatty Acid + H_2O_2

$2H_2O_2$ + 4-aminoantipyrine + TODB $\xrightarrow{\text{peroxidase}}$ Quenone + $5H_2O$

3.6.4.4 Triglyceride Determination

Principle and Methodology

The present method for triglyceride determination uses a modified Trinder (Trinder, 1969; Barham and Trinder, 1972) colour reaction to yield a fast, linear, endpoint reaction (Fossati and Prencipe, 1982; McGowan *et al.*, 1983). Triglycerides in the sample are hydrolyzed by lipase to glycerol and fatty acids. The glycerol is then phosphorylated by adenosine-5-triphosphate (ATP) to glycerol-[1]-phosphate (G-[2]-P) and adenosine-5diphosphate (ADP) in a reaction catalyzed by glycerol kinase (GK). G-3-P is then converted to dihydroxyacetone phosphate (DAP) and hydrogen peroxide by glycerophosphate oxidase (GPO). The hydrogen peroxide then reacts with 4aminoantipyrine (4-AAP) and 3, 5-dichloro-2-hydroxybenzen (3, 5-DHBS) in a reaction catalyzed by peroxidase to yield a red coloured quinoneimine dye. The intensity of the colour produced is directly proportional to the concentration of triglycerides in the sample.

Triglycerides + H_2O lipoprotein lipase Glycerol + Fatty acids

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[1] ***.6.4.5 Low Density Lipoprotein Cholesterol Determination***

LDL was calculated according to Friedwald's formula (Friedwald *et al.*, 1972).

LDL-Cholesterol (mmol/l) = Total Cholesterol –HDL-Cholesterol-Triglyceride/2.2

[2] **.6.4.6 Very Low Density Lipoprotein Cholesterol Determination**

VLDL-C was calculated according to the formula proposed by Wilson (DeLong *et al.*, 1986) in accordance with the manufacturer's instructions;

Glycerol + ATP $\xrightarrow{\text{Glycerol kinase}}$ Glycerol-3-Phosphate + ADP

Glycerol-3-Phosphate + O_2 $\xrightarrow{\text{GPQ}}$ DAP + H_2O_2

H_2O_2 + 4-aminoantipyrine + p-Chlorophenol $\xrightarrow{\text{peroxidase}}$ Quinoneimine

VLDL= Triglycerides/5 or 0.2 x Triglycerides

3.6.4.7 Hs-CRP Determination *Principle and methodology i*-CHROMA™ hsCRP is based on fluorescence immunoassay technology. It uses a sandwich immunodetection method, such that by mixing detector buffer with blood specimen in test vial, the fluorescence-labeled detector anti-CRP antibody in buffer binds to CRP antigen in blood specimen. As the sample mixture is loaded onto the sample well of the test device and migrates the nitrocellulose matrix of test strip by capillary action, the complexes of detector *i*-CHROMA™ hsCRP antibody and CRP are captured to antiCRP sandwich pair antibody that has been immobilized on test strip. Thus the more CRP antigen is in blood specimen, the more complexes are accumulated on test strip. Signal intensity of fluorescence of detector antibody reflects amount of CRP captured and is micro processed from *i*-CHROMA™ Reader to show CRP concentration in blood specimen. The default result unit of *i*-CHROMA™ hsCRP is displayed as an mg/L from *i*CHROMA™ Reader. The working range and the detection limit of system are 0.1-10 mg/L. and 0.1 mg/L, respectively.

3.6.4.8 IL-6 and TNF-α Determination

Principle and methodology

Serum IL-6 and TNF-α levels were determined using the FluoroSpot technique. The principle objective is the simultaneous measurement of dual cytokine secretion at the single cell level. This is accomplished by using a mixture of monoclonal antibodies (mAbs); biotinylated detection mAb for one analyte, tag-labeled detection mAb for the other analyte and fluorophore-labeled detection reagents, which enable analysis of several cytokines or other secreted analytes in the same well. The detection step is amplified by secondary addition of Streptavidin conjugated to a red fluorophore and an anti-tag mAb labeled with a green fluorophore. Spot analysis is performed with an automated fluorescence reader generating separate images for the two fluorophores. Two-colored spots, derived from cells secreting dual cytokines are identified by an analysis of co-positioned spots in a digital overlay of the single stain images.

3.6.5 Analysis of haematological parameters

EDTA.K3 anticoagulated whole blood was run on haematology automated analyser where Total WBC counts were obtained among other parameters using cell pack reagents and stromatolysers from Sysmex Corporation, Japan. ESR levels obtained using Westergren ESR kits (Guest Scientific Swaziland).

3.6.5.1 Erythrocyte Sedimentation Rate Determination

Principle and Methodology

Sedimentation of red cells is affected by forces both for and against sedimentation. The forces resisting sedimentation are the negative charge on the red cell surface (causing red cells to repel each other (zeta potential), the up flow of plasma displaced by falling red cells, and the rigidity of red cells. The forces accelerating sedimentation are anemia and plasma proteins. Plasma proteins bind to red cell membranes thereby reducing the zeta potential thus allowing rouleaux formation to occur. The Westergren method employs a 200 mm, 2.5 mm diameter tube vertically aligned column. The column is filled with blood anticoagulated. The distance that the column of blood falls in one hour is recorded and reported in mm/ at the end of 1st hour (Hameed and Waqas, 2006).

3.6.5.2 Automated WBC counting

Principle and Methodology

The Sysmex KX-21N automated analyser uses stromatolyser as a lysing agent to induce haemolysis of erythrocyte and the formation of ultramicroscopic pore in the leukocyte cell membrane. A fluorescent dye is introduced through the pores into the cells to stain nucleic acids and organelles. The leukocytes sensitised to the reagents are detected by flow cytometry. By applying electric impedance (DC) and electric capacitance (RF)

methods cell volume and internal information can be estimated.

3.7 STATISTICAL ANALYSIS

Categorical data was presented as figure with percentage in parenthesis. Student's unpaired t-test was used to compare mean values of group continuous data. A p-Value <

0.05 was taken as significant. Parametric data was presented as means ± standard deviation of the mean. Non parametric data was expressed as geometric mean (95% CI of geometric mean). Where appropriate, continuous data were compared using unpaired t-test& Mann-Whitney and one-way ANOVA with a Bonferroni posttest or

Kruskal-Wallis with Dunn's posttest.

A p-Value < 0.05 was taken as significant. Graph Pad Prism version 6.00for windows (Graph pad software, San Diego California, USA, www.graphpad. Com) and IBM Statistical Package for the Social Sciences (SPSS Inc, Chicago, USA; (www.spss.com) version 20.00 were used for data analysis where appropriate.

The percentage risk of developing coronary heart disease over the next 10 years (Absolute Risk) was determined on the Framingham Risk Score (Brindle *et al.*, 2003) comparing the risk scores to the risk of others of the same age (Relative Risk). Those with percentage risk score equal or below the low risk population were classified as low risk, those with risk scores above the low risk group of same age but greater than the average risk group of the same age were classified as having moderate 10 year risk while participants with percentage scores over and above the average risk of same group were considered as

having high risk.

3.8 ETHICAL ISSUES

Ethical clearance for commencement of the study was sought from the Committee on Human Research, Publication and Ethics (CHRPE), Kwame Nkrumah University of

Science and Technology, School of Medical Sciences (KNUST-SMS), & Komfo Anokye Teaching Hospital (KATH), Kumasi, Ghana. All procedures were approved by the Ethics Committee of the School of Medical Sciences, KNUST and KATH, Kumasi

(*CHRPE*/*RC*/119/12). A written informed consent form was completed by all the participants who were recruited into the study. The study was explained to the subjects in English and/or, Ewe, Ga, and Twi languages. Pretested questionnaires were used to record socio-demographic information, medical and family history of the subjects. The folders of the case subjects were reviewed to ascertain the medications they were on. Thorough general physical examinations were conducted on these subjects by the clinician.

CHAPTER FOUR

RESULTS

Out of the study population of 187, participants classified as control were 62(33.2%) and the rest as case presenting with diabetes, hypertension or both 125(66.8%). As can be seen from table 1, majority of the participants were females 130(69.5%). In general significantly higher levels of education were reported among the controls, the inverse was however observed among the case group. Majority of the respondents worked within one (1) to eight (8) hours a day, though longer working hours was recorded among the case group in comparison to the control (p-0.0415). Dietary salt, sugar and fat intake as well as alcohol intake was predominantly moderate among the study group with consumption significantly higher toward the controls. Casual participation in exercise was recorded among respondents; with significant majority of the case group not engaged at all in exercise 67(54.0%). Table 1.

Table 4.1: General Socio-Demographic Characteristics of Respondents Stratified by Disease Status

Parameter	Total n-187	Control n-62	Case n-125	P-value
Gender				
Female	130(69.5)	43(69.4)	87(69.6)	0.5505
Male	57(30.5)	19(30.7)	38(30.4)	
Educational Background				
None	45(24.1)	6(9.7)	39(31.2)	< 0.0001
Basic	68(36.4)	11(17.7)	57(45.6)	
Secondary	23(13.4)	9(14.5)	17(13.6)	
Tertiary	47(11.0)	36(58.1)	12(9.6)	
Working Hours				
None	37(19.8)	8(12.9)	29(23.2)	0.0415
1-8 Hours	115(61.5)	47(75.8)	68(54.4)	
>8 Hours	35(18.7)	7(11.3)	28(22.4)	
Dietary Salt				
Moderate	165(88.2)	57(91.9)	108(86.4)	0.0134
High	9(4.8)	5(8.1)	4(3.2)	
Dietary Sugar				
Moderate	107(57.2)	55(88.7)	52(41.6)	< 0.0001
High	10(5.3)	6(9.7)	4(3.6)	
Dietary Fat				
Moderate	147(78.6)	52(83.9)	95(76.0)	0.0003

High	14(7.5)	9(14.5)	5(4.0)	
Alcohol Intake				
Moderate	54(28.9)	32(51.6)	22(17.6)	< 0.0001
High	11(5.9)	3(4.8)	8(6.4)	
Exercise				
None	88(47.3)	21(33.9)	67(54.0)	0.0266
Not Often	73(39.2)	32(51.6)	41(33.1)	
Very Often	25(13.4)	9(14.5)	16(12.9)	
Family History H,D,H/D				
One				
	81(43.3)	32(51.6)	49(39.2.0)	0.1914
More Than 1	46(24.6)	11(17.7)	35(28.0)	

Data is presented as figure with percentage in parenthesis. P is significant at 0.05. n- number, H- Hypertension, Diabetes, H/D - Both Hypertension and Diabetes.

Table 4.2: General Anthropometric, Hemodynamic, Haematological and Biochemical Characteristics of Study Population Stratified by Disease Status.

Parameter n-187	**Total**	**Control n-62**	**Case n-125**	**p-value**
Age (years)	48.2±14.2	47.3±15	50.4±13.4	0.1541
Anthropometric Parameters				
Weight (kg)	71.6±16.4	69.2±15.7	73.0±17.9	0.1393
Height (m)	1.6±0.01	1.7±0.01	1.6±0.01	0.1049
BMI (kg/m^2)	26.9±5.5	25.6±5.5	27.7±6.7	0.0181
WC (cm)	87.7±14.1	79.2±11. 8	92.5±12.3	< 0.0001
HC (cm)	102.5±15. 5	98.1±11. 8	104.9±16.8	0.0025
WHR	0.86±0.01	0.81±0.01	0.89±0.01	< 0.0001
Hemodynamic Parameters				
SBP (mmHg)	131±26	113.9±12. 6	140.7±26.8	< 0.0001
DBP (mmHg)	80.9±13. 7	73.7±8.7	85.0±13.4	< 0.0001

Haematological Assays				
HB(g/dl)	12.5±0.1	12.9±0.2	12.3±0.2	0.0428
WBC(10^9/L)	5.5±1.4	4.9±1.5	5.8±1.1	< 0.0001
ESR(mmfall/hr)	47.3±15.3	40.9±10.5	51.0±20.1	0.0157
Biochemical Assays				
FBG(mmol/l)	8.1±0.4	5.3±0.1	9.7±0.5	< 0.0001
TC(mmol/l)	5.2±0.1	4.5±0.1	5.5±0.1	< 0.0001
TG(mmol/l)	1.4±0.1	1.1±0.1	1.6±0.1	0.0004
HDL-C(mmol/l)	1.6±0.04	1.4±0.04	1.7±0.1	0.0002
LDL-C(mmol/l)	2.9±0.1	2.6±0.1	3.1±0.1	< 0.0001
VLDL-C(mmol/l)	0.3±0.01	0.2±0.02	0.3±0.01	0.0004
Hs-CRP(mg/L)	1.2(0.8-1.7)	0.3(0.2-0.5)	1.3(0.8-1.9)	0.0007
IL-6(pg/ml)	13.7(10.2-18.6)	12.0(6.7-21.7)	14.7(10.4-20.9)	0.5820
TNF-α(pg/ml)	10.1(5.6-18.4)	20.2(11.0-37.2)	12.5(6.8-22.9)	0.4208

Parametric data is presented as means ± standard deviation of the mean and nonparametric data as geometric mean (95% CI of geometric mean. P is significant at 0.05.Continuous data were compared using unpaired t-test& Mann-Whitney where appropriate. HB: Haemoglobin, WBC: White Blood Cell, ESR: Erythrocyte Sedimentation Rate, VLDL-C: Very Low Density Lipoprotein-Cholesterol, LDL-C: Low Density Lipoprotein-Cholesterol, HDL-C: High Density Lipoprotein, TC: Total Cholesterol, TG: Triglycerides, FBG: Fasting Blood Glucose, WHR: Waist-to-Hip Ratio, BMI: Body Mass Index, WC: Waist Circumference, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, IL: Interleukin 6, TNF-A: Tumor Necrotic Factor Alpha.

The average age of the respondents in this study was 48.2. In general the anthropometric parameters of the case group was significantly higher compared to the controls, the exception though was found in the height of participants where though not statistically significant the controls were averagely 0.1 meter taller than the cases. Significantly higher systolic and diastolic blood pressures values were observed among the cases compared to the controls. The average WBC and ESR levels serving as proxy markers for nonspecific inflammations were significantly higher among the case group. Among the biochemical markers assayed, higher glycaemic and atherogenic lipid levels were

observed among the cases. The median Hs-CRP concentration was also higher in the case group. Table 2.

Table 4.3: General Anthropometric, Hemodynamic, Haematological and Biochemical Characteristics of Cases Stratified by Disease Type

Parameter n-40	Diabetes	Hypertension n-43	Diabetes/Ht n-42	p-value
Age	48.3±13.9	57.1±12.5**	58.2±12.3**	0.0009
Anthropometric Paramete rs				
Weight (kg)	67.5±14.5	72.2±16.4	75.4±16.5	0.0910
Height (m)	1.6±0.6	1.6±0.0	1.6±0.0	0.6341
BMI (kg/m²)	25.4±6.3	27.2±5.2	28.7±5.8*	0.0415
WC (cm)	85.2±13.3	90.6±11.1 ¥	97.8±12.3***	<0.0001
HC (cm)	97.9±13.9	103.9±14.4	108.7±17.5**	0.0080
WHR	0.9±0.6	0.9±0.0	0.9±0.0	0.0669
Hemodynamic Parameters				
SBP (mmHg)	119.0±15.2	149.8±23.6***	143.8±23.9***	< 0.0001
DBP (mmHg)	74.9±9.5	89.8±11.1** *	86.0±12.3***	< 0.0001
Haematological Assays				
HB(g/dl)	12.6±1.9	12.7±1.9	11.9±1.3	0.0575
WBC(10^9/L)	5.8±1.9	5.4±1.3	6.1±1.3	0.0837

ESR(mmfall/hr)	49.3±15.1	49.0±14. 7	52.7±14.8	0.8047
Biochemical Assays				
FBG(mmol/l)	12.4±4.4	6.3±2.6***¥¥¥	11.5±2.7	< 0.0001
TC(mmol/l)	5.1±1.2	5.6±1.3	5.6±1.3	0.1030
TG(mmol/l)	1.5±0.6	1.6±0.7	1.5±0.6	0.9148
HDL-C(mmol/l)	1.5±0.6	1.7±0.7	1.7±0.6	0.0807
LDL-C(mmol/l)	3.0±1.2	3.2±0.6	3.2±0.6	0.5243
VLDL-C(mmol/l)	0.4±0.1	0.3±0.0*	0.3±0.0*	0.0069
Hs-CRP(mg/L)	3.4(1.9-5.8)a	1.6(0.8-3.3)a	1.1(0.6-2.3)a	<0.0001
IL-6(pg/ml)	10.1(5.6-18.4)	20.2(11.0-37.2)	12.5(6.8-22.9)	0.6595
TNF-α(pg/ml)	62.9(34.7-114.2)	51.9(33.3-80.9)	63.9(43.8-93.5)	0.6967

Data is presented as means±standard deviation of the mean and median(interquartile range). P is significant at 0.05. Continuous data were compared using one-way ANOVA with a Bonferroni posttest or Kruskal-Wallis with Dunn's posttest where appropriate. Ht: Hypertensives, HB: Haemoglobin, WBC: White Blood Cell, ESR: Erythrocyte Sedimentation Rate, VLDL-C: Very Low Density Lipoprotein-Cholesterol, LDL-C: Low Density Lipoprotein-Cholesterol, HDL-C: High Density Lipoprotein, TC: Total Cholesterol, TG: Triglycerides, FBG: Fasting Blood Glucose, WHR: Waist-to-Hip Ratio, BMI: Body Mass Index, WC: Waist Circumference, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, IL: Interleukin 6, TNF-A: Tumor Necrotic Factor Alpha. P- compares all three, [a] compares control with any, * compares of hypertensive or diabetic/hypertensive with diabetes, ¥ compares of hypertensive and diabetic/hypertensive.

Significant intra group age differences was observed among the cases, with the mean age increasing from those presenting with diabetes (48.3±2.2) through those presenting with hypertension (57.1±1.9) to subjects presenting with both conditions (58.2±1.9). Significant differences were also observed among the case with respect to both waist and hip circumference, presenting a trend as afore mentioned for age. Patients presenting with both diabetes and hypertension had the least haemoglobin concentration but highest levels of WBC as well as ESR. The highest average fasting plasma glucose concentration was reported among the diabetic only group (12.4±0.7) followed by those with both chronic conditions (11.9±0.2). Lipid indices as well as the inflammation biomarker assayed in this study were found to be comparable across the case group, with the exception of Hs-CRP protein where significant intra group differences were observed.

Table 3.

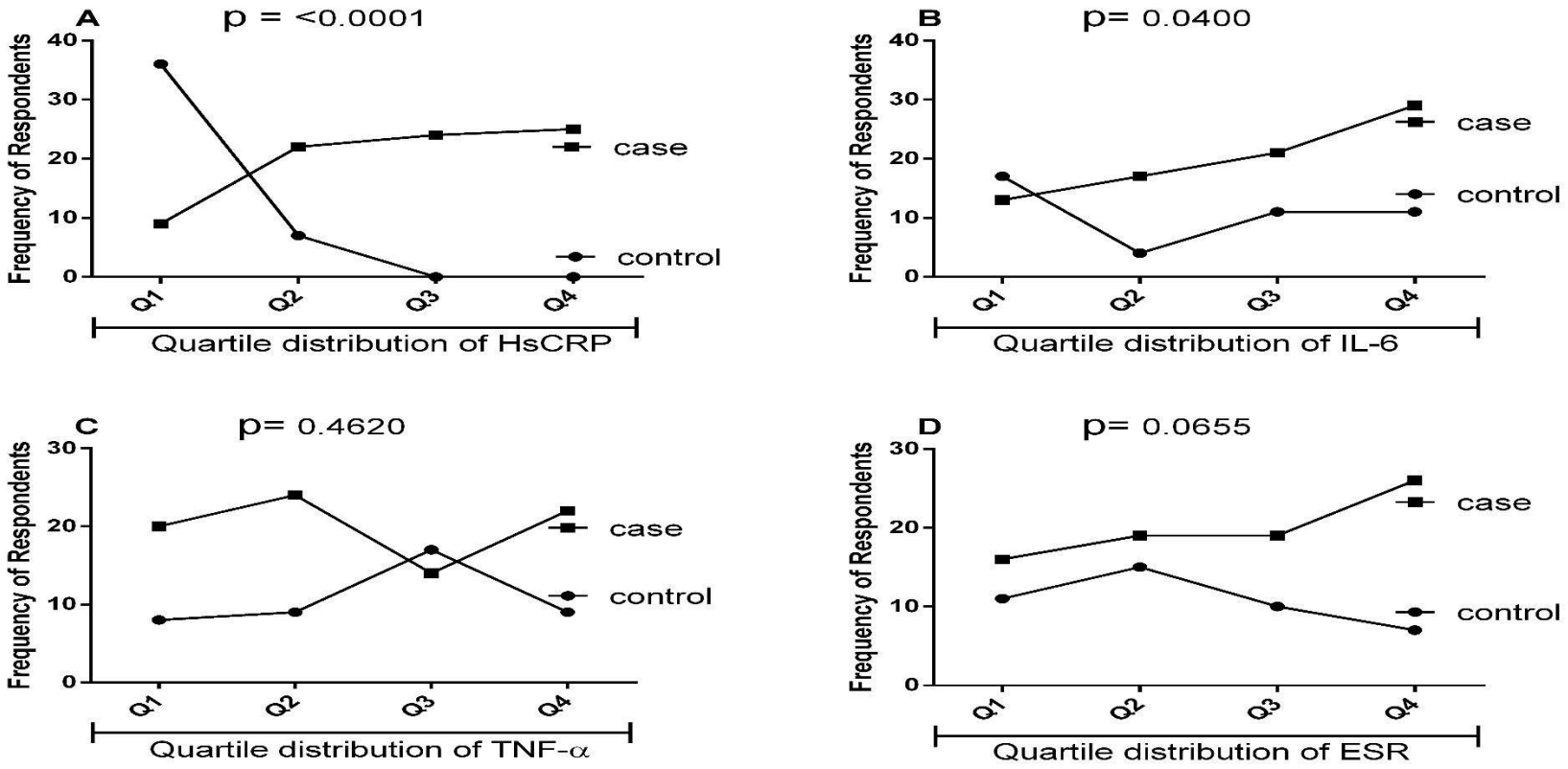

Figure 4.3: Trends of female quartile cluster distributions of respondents by inflammation biomarkers stratified by case and control. Q1 - First quartile, Q2 - Second quartile, Q3 - Third quartile, Q4 - Fourth quartile. HsCRP- High sensitive Creactive protein, IL-6- Interleukin 6, TNF-α- Tumor Necrotic Factor Alpha, ESR- Erythrocyte sedimentation rate

Among the female populations, the control groups exhibited a significantly different quartile trend of high sensitive C-reactive protein levels compared to the case group. Thus majority of the controls 83.73% clustered at the first quartile of high sensitive Creactive protein concentration with the remaining 16.28% in the second. However the population cluster among the cases increased from the first through to the fourth quartile of high sensitive C-reactive protein concentration 11.25%, 27.50%, 30.00% and 31.25% from the first to the fourth quartiles respectively) (fig 1A).

The trend clusters of female study population for Interleukin 6 levels saw a steady increase in frequency from the first through to the fourth quartile among the case but a 39.54% initial number cluster below the 25th percentile cutoff reduced to 25.58% beyond the 50th percentile cutoff of Interleukin 6. (Fig 1B). As seen in figure 1C, no significant distinguishable trends were observed among the case and the control groups when study participants were grouped with the levels of tumor necrotic factor alpha (p-0.4620). Though not statistically significant a continuous increasing number of participants among the case group was found with increasing fall in erythrocyte whiles the opposite was generally observed among the control group. (figure 1D).

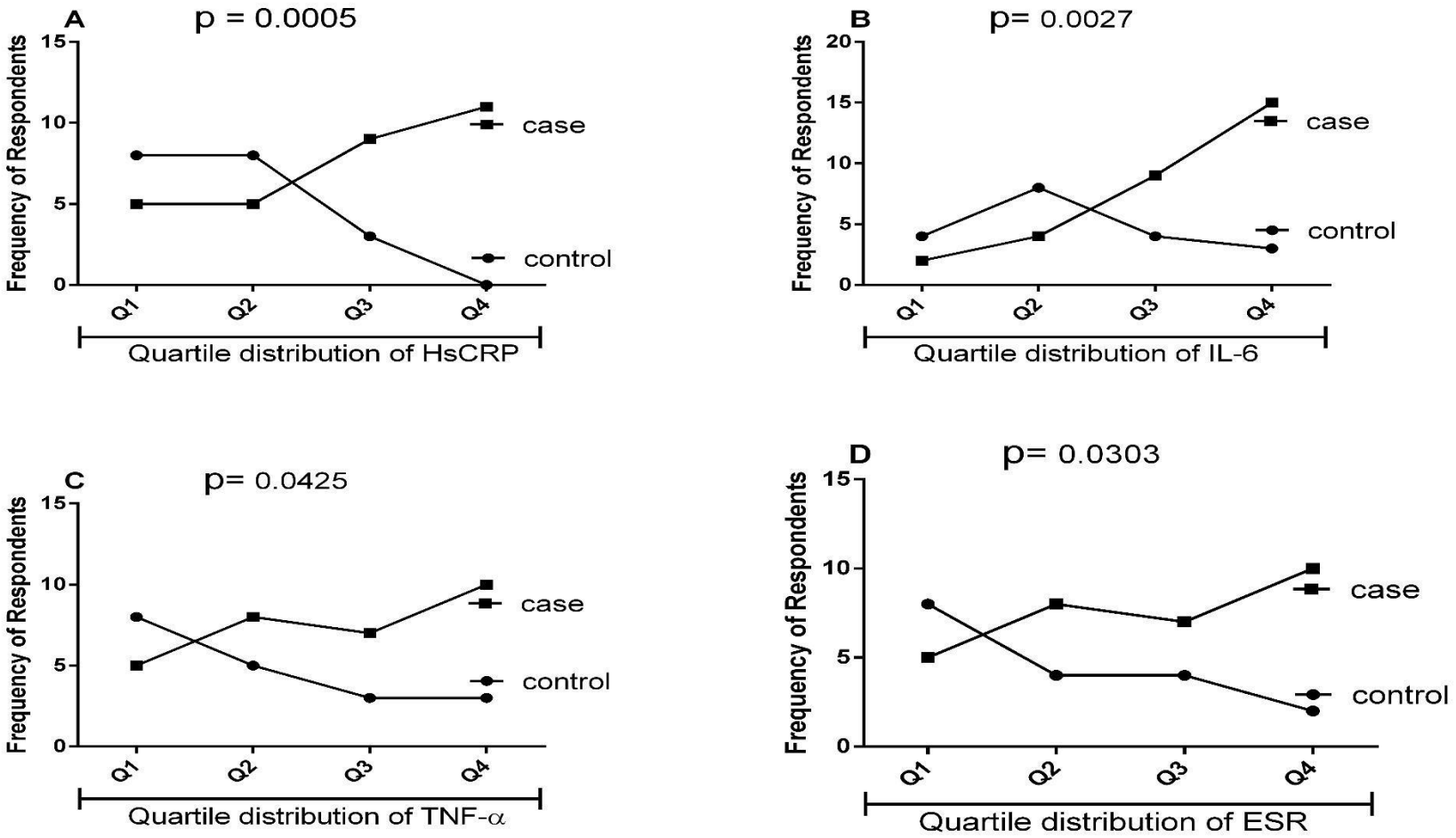

Figure 4.4: Trends of male quartile cluster distributions of respondents by inflammation biomarkers stratified by case and control. Q1- First quartile, Q2-Second quartile, Q3-Third quartile, Q4-Fourth quartile. HsCRP- High sensitive C-reactive protein, IL-6-Interleukin 6,TNF-α-Tumor Necrotic Factor Alpha, ESR- Erythrocyte sedimentation rate.

As can be seen from figure 2, the trends of population cluster in general recorded significant increasing numbers of patients (case group) from the first to the fourth quartile whilst the inverse was observed among the control group. Out of a total of 19 male participants in the control group 16 representing 84.21% had hs-CRP levels lower than the second quartile cutoff, whiles 20 out of the total male case population of 38 patients had hs-CRP levels over the cutoff of the second quartile (fig 2A).With IL-6 the percentage cluster trend was (6.67%, 13.33%, 30.00%, and 50.00% for first to fourth quartile respectively) for the case group and (21.05%, 42.11%, 21.05% and 15.79% for first to fourth quartile respectively) for the control group. That of TNF- α was for case (16.67%, 26.67%, 23.33% and 33.33% for first to fourth quartile respectively) and control (42.10%, 26.32%, 15.79% and 15.79% for first to fourth quartile respectively). The ESR percentage cluster trend for the case group was (16.67%, 26.67%, 23.33% and 33.33% for first to fourth quartile respectively) and (47.37%, 21.05%, 21.05% and 10.53% for first to fourth quartile respectively) for the control group.

Table 4.4: Pearson's correlation coefficients of Anthropometric variables, Haemodynamic, Haematologic , Atherogenic and inflammation Indices for control group (upper right-hand side) and case group (lower left-hand side).

Parameter	BMI	WC	HC	WHR	SP	DP	HB	WBC	ESR	FBG	TC	TG	HDL	LDL	VLDL	hsCRP	IL6	TNFA
BMI		.71**	.66**	-.03	.11	.18	.01	-.05	.01	.014	.08	.14	-.06	.08	.14	.51**	-.07	-.20*
WC	.68**		.75**	.29**	.19*	.21*	.06	.02	-.06	.058	.08	.17	-.11	.09	.17	.13	-.10	-.14
HC	.81**	.82**		-.41**	.16	.19	.01	-.02	.06	.067	.03	.15	-.14	.06	.15	.18	-.12	-.15
WHR	.07	.63**	.06		.05	.01	.05	.10	-.14	-.017	.06	-.01	.05	.05	-.01	-.13	.03	.03
SP	.33**	.39**	.27*	.31*		.70**	.03	.09	.05	-.190	-.01	.03	-.02	-.01	.03	-.11	.07	.09
DP	.38**	.43**	.35**	.26*	.83**		.05	-.03	-.01	-.155	-.11	.05	-.17	-.05	.05	-.17	-.03	.03
HB	.04	.14	.03	.18	.14	.12		-.06	-.49**	-.139	-.07	.09	-.20*	.01	.09	-.16	-.08	-.05
WBC	.15	.28*	.13	.33**	.10	.03	-.24		.26**	.018	.12	.02	.04	.11	.02	.39*	.14	-.01
ESR	.15	.12	.17	-.05	.06	.02	-.56**	.18		.149	.06	-.11	.14	.02	-.11	.52**	.05	-.15
FBG	.15	.11	.23	-.13	.27*	.27*	.25	-.11	-.15		-.03	.10	-.06	-.04	.10	.21	.31**	-.05
TC	.10	.12*	.16	.15	.40**	.32*	.05	.01	.15	.276*		.19*	.51**	.79**	.19*	.26	.10	-.03
TG	-.04	-.23*	-.05	.03	-.04	-.02	.05	-.02	-.13	.031	.30*		-.10	-.14	1**	-.01	-.11	-.07
HDL	-.17	-.37**	-.06	-.03*	.14	.01	-.25	-.08	.18	.038	.52**	.20		.01	-.10	.34*	.14	.02
LDL	.21	.29*	.24	.18	.42**	.38**	.14	.06	.17	.287*	.78**	-.24	.06		-.14	.08	.08	-.02
VLDL	.04	.01*	.05	.03	-.04	-.02	.05	-.02	-.13	.031	.30*	1**	.20	-.24		-.01	-.11	-.07
hsCRP	.83**	.59*	.78**	.08*	.67*	.67*	-.21	.41	.27	.583*	.02	-.02	-.08	.06	-.02		.04	-.01
IL6	.01	.11	.07	.09	-.02	-.05	.03	-.05	-.19	.227	.25	.10	.06	.21	.10	-.24		.06
TNF- α	.03	.19	.06	.27	.21	.27	-.12	.27	.20	-.08	.30	-.03	.17	.28	-.03	-.07	.12	

HB: Haemoglobin, WBC: White Blood Cell, ESR: Erythrocyte Sedimentation Rate, VLDL-C: Very Low Density Lipoprotein-Cholesterol, LDL-C: Low BMI: Body Mass Index, WC: Waist Circumference, HC: Hip Circumference, WHR: Waist-to-Hip Ratio, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, HB: Haemoglobin, WBC: White Blood Cell, ESR: Erythrocyte Sedimentation Rate, FBG: Fasting Blood Glucose , TC: Total Cholesterol, TG: Triglycerides, HDL: High Density Lipoprotein, LDL: Low Density LipoproteinCholesterol, VLDL: Very Low Density Lipoprotein-Cholesterol, HsCRP- High sensitive C-reactive protein, IL: Interleukin 6, TNF-A: Tumor Necrotic Factor Alpha. *.Correlation is significant at the 0.05 level (2-tailed), **.Correlation is significant at the 0.01 level (2-tailed), ***.Correlation is significant at the 0.001 level (2-tailed)

In the control group the study revealed no significant correlation among the anthropometric variable with the haematologic, atherogenic, as well as glycaemic indices. An observed increase in waist circumference corresponded to an increase in both the systolic and diastolic blood pressure. Increase body mass index resulted in a significant increase in hs-CRP and decrease in TNF-α levels among the control group. Among the case group an increase in any of the anthropometric indices (BMI,WC,HC,WHR) was associated with a corresponding increase in the haemodynamic parameters measured for this study (SBP and DBP). Positive correlation was observed among WC as well as WHR and WBC. WC was associated with all the atherogenic indices assayed, whiles an increased in any of the anthropometric variables saw a corresponding increase in hsCRP among the case group.

The haemodynamic parameters of the case group were found to be positively associated with glyceamia, TC and LDL and most strongly with hs-CRP. WBC and ESR significantly correlated positively with hs-CRP among the control group but this relation was not significant in the case group. For the control participants significant association was recorded between glyceamic levels and IL-6. In the patients the relation was between glycaemic levels with TC, LDL and hs-CRP. Table 4.

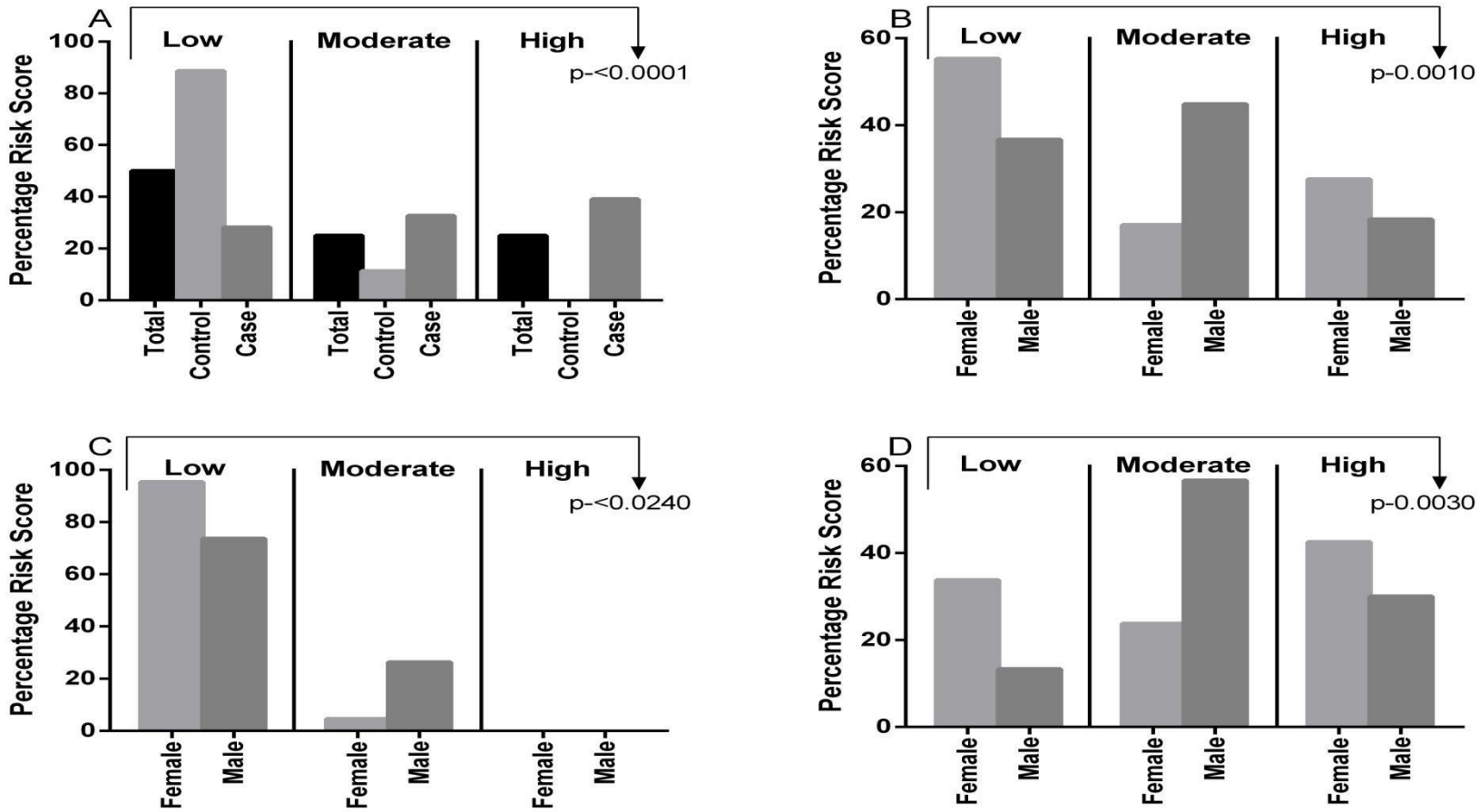

Figure 4.5: Relative risk scores of Framingham percentage risk of heart disease in ten year, stratified by case, control and gender. A-Case and Control, B-Gender, C-Control only, D-Case only.

In the general study population it was observed that 25% of the subjects had a high risk of developing coronary heart disease over the next ten years. A further 25% were estimated to have moderate risk of developing coronary heart disease within the next ten years. As shown figure 3A, significantly all the subjects classified as having high risk of developing coronary heart disease were of the case group (39.1%). Additionally 32.7% of the case group was estimated to carry a moderate risk of developing coronary heart disease compared to 11.3% of the control group. Gender variations in percentage ten year risk estimations was observed, with significantly greater proportion of female participants than male exhibiting high risk profile and the reverse observed at the moderate risk cluster.Figure3B&D.

Table 4.5: Framingham percentage risk of heart disease in ten year, stratified by case, control and levels of inflammation markers

Parameter			Low	Moderate	High	p-Value
Tumor Necrotic Factor-α	Q1	Control	13(100)	0(0.0)	0(0.0)	< 0.0001
		Case	7(20.6)	16(47.1)	11(32.4)	
	Q2	Control	10(83.3)	2(16.7)	0(0.0)	0.0072
		Case	13(36.1)	6(16.7)	17(47.2)	
	Q3	Control	21(95.5)	1(4.5)	0(0.0)	< 0.0001
		Case	9(34.6)	8(30.8)	9(34.6)	
	Q4	Control	11(73.3)	4(26.7)	0(0.0)	0.0026
		Case	7(24.1)	11(37.9)	11(37.9)	
Interleukin-6	Q1	Control	27(90.0)	3(10.0)	0(0.0)	< 0.0001
		Case	18(32.1)	20(35.7)	18(32.1)	
	Q2	Control	3(75.0)	1(25.0)	0(0.0)	0.0498
		Case	0(0.0)	0(0.0)	2(100)	
	Q3	Control	13(92.9)	1(7.1)	0(0.0)	< 0.0001
		Case	6(17.6)	14(41.2)	14(41.2)	
	Q4	Control	12(85.7)	2(14.3)	0(0.0)	0.0023
		Case	11(33.3)	7(21.2)	15(45.5)	
High Sensitive C-Reactive Protein	Q1	Control	33(86.8)	5(13.2)	0(0.0)	< 0.0001
		Case	0(0.0)	10(62.5)	6(37.5)	
	Q2	Control	10(52.6)	9(47.4)	0(0.0)	0.0091
		Case	12(42.9)	6(21.4)	10(35.7)	
	Q3	Control	5(100)	0(0.0)	0(0.0)	0.017
		Case	13(33.3)	10(25.6)	16(41.0)	
	Q4	Control	0(0.0)	0(0.0)	0(0.0)	*Nd*
		Case	10(23.8)	16(38.1)	16(38.1)	

Data is presented as frequency with corresponding percentages in parenthesis. Q1-First quartile, Q2-Second quartile, Q3- Third quartile and Q4- fourth quartile, nd- not done

The odds of developing coronary heart disease within ten years was in general significantly higher among the case group compared to the control across the various categorization of levels of inflammation markers assayed in this study.(Table5)

CHAPTER FIVE

DISCUSSION

Working for long hours has been identified as a potential work-related risk factor for ill health and persons working longer hours are more likely to be exposed to high job demands hence would have less time for recreation than their counterparts who work fewer hours (Virtanen *et al.*, 2012). Prolonged exposure to psychological stress at work is a deterioration of lifestyle linked to the risk of diabetes (Kuwahara *et al.*, 2014). Virtanen *et al.* (2012) in a prospective study reported that approximately 40% excess risk of CHD is conferred on employees working for long hours. The observation of prolonged working hours associated with diabetes and cardiovascular morbidity was confirmed in the current study. Though majority of the respondents worked within one (1) to eight (8) hours a day, significant difference in the length of period of work was observed among the case and the control groups (p-0.0415) with longer working hours exhibited by the case group compared to the control.

In the present study, dietary salt, sugar, fat as well as alcohol intake was predominantly moderate among the study group with higher consumption significantly tilted toward the controls. In contrast to the findings of this study, Breen *et al.* (2014) reported higher intake of dietary fat among type 2 diabetic subjects in a comparative analysis study of nutrient intake with or without type

2 diabetes. A study done among persons presenting with hypertension in an urban South Indian Population, found higher consumption of dietary sugar and salt (Radhika *et al.*, 2007). These observations also contrasted with those obtained in this study as greater consumptions was observed among control participants compared to the case counterparts. Population-based prospective studies regarding alcohol consumption however have recorded inconsistent results. Spranger *et al.* (2003) observed higher consumption among persons with type 2 diabetes than those without diabetes whiles consistent with this study, the intake of alcohol was greater among healthy control subjects than their counterparts presenting with type 2 diabetes and hypertension (Hu *et al.*, 2004; Liu *et al.*, 2007; Sesso *et al.*, 2007). The effects of poor dietary consumption as potential risk factors for morbidity among apparently healthy subjects have been documented in some studies (Mensink and Katan, 1990; Bantle *et al.*, 2000; Hu *et al.*, 2001). WHO/FAO (2003) reported that, high intake of saturated dietary fat and salt (sodium) are linked to elevated blood pressure, a major risk factor for stroke and coronary heart disease. In a previous study, Nakanishi *et al.* (2001), observed that diets with high glycaemic load and low cereal fibre content combined with psychological factors are associated with the risk of type 2 diabetes. Heavy alcohol consumption was found to be associated with diseases like stroke, alcoholic cardiomyopathy and hypertension (Pearson *et al.*, 2003).

The current study recorded a casual participation in exercise among respondents; with a significant majority of the case group not engaged at all in exercise 67(54.0%).The finding from this study is in tandem with results of various prospective studies in which type 2 diabetes and hypertensive subjects significantly exercised less compared to controls (Spranger *et al.*, 2003; Hu *et al.*, 2004; Liu *et al.*, 2007; Sesso *et al.*, 2007). The present study reaffirms the observation that physical inactivity or sedentary lifestyle is a common phenomenon with 60% to 85% of people in the world from both developed and developing countries lead sedentary lifestyles (Seabra *et al.*, 2008). Cohort studies have suggested that physical activity plays a role in the reduction of incident type 2 diabetes and CHD (Bassuk and Manson, 2005). Results of 8 years longitudinal Nurses' Health Study showed that brisk walking for at least 2.5 hours per week was associated with 25% reduction in diabetes after adjustment for age, body mass index (BMI) and other risk factors for diabetes (Hu *et al.*, 1999). Similarly, one hour per week walking was associated with 50% reduction in CHD risk in the Women's Health Study (Lee *et al.*, 2001).

Generally, the anthropometric parameters of the case group were significantly higher compared to the controls. The difference in the waist and hip circumferences between type 2 diabetes, hypertension or participants presenting with both conditions was significant. Among the case subjects, all the anthropometric indices (BMI, WC, HC, and WHR) were positively

associated with haemodynamic parameters measured for this study (SBP and DBP). Consistent with the current findings, earlier studies have reported significantly higher measures of obesity and adiposity in type 2 diabetes and hypertension (Hu *et al.*, 2004; Pai *et al.*, 2004; Owiredu *et al.*, 2008; MarquesVidal *et al.*, 2012). In a prospective study to examine the relationship between body fat distribution and 2-year incidence of hypertension and stroke indicated that, hypertension was related to abdominal adiposity in a cohort of 41,837 subjects (Selby, 1989). Findings from the Health Professionals Follow-Up Study also revealed a predictive risk association between increasing quintile cluster of waist circumference and diabetes after age-adjusted characteristics (Wang *et al.*, 2005). A Pentecostal population in the Kumasi metropolis recorded a close association between hypertension and central obesity (waist circumference) (Owiredu *et al.*, 2008). The positive associations between adiposity and elevated blood pressures reported by the above authors were confirmed in the current study. Mechanisms linking obesity to haemodynamic dysregulation are still areas under intense scrutiny. However, one author indicates that overweight and obesity are associated with adipose tissue dysfunction, characterized by enlarged hypertrophied adipocytes, increased infiltration by macrophages and marked changes in secretion of adipokines and free fatty acids resulting in chronic vascular inflammation, oxidative stress, activation of the reninangiotensin-aldosterone system and sympathetic overdrive (Dorresteijn

et al., 2012). During the early phases of obesity, primary sodium retention may exists as a result of increase in renal tubular reabsorption resulting in expansion of extracellular-fluid volume and the kidney-fluid apparatus resetted to a hypertensive level consistent with a model of hypertension because of volume overload (Kotsis *et al.*, 2010).

In this study, with the exception of HDL-C, the case subjects exhibited significantly higher atherogenic lipid levels than the controls. Also the haemodynamic parameters of the case group were found to be positively associated with glyceamia, TC and LDL-C. Earlier studies from various populations have reported findings agreeing with the results of this study where higher levels of unfavourable lipid profiles were recorded in type 2 diabetes and hypertensive subjects. (Idogun *et al.*, 2007; Pitsavos *et al.*, 2007; Gordon *et al.*, 2010; Agrawal *et al.*, 2014; Idemudia, 2014). HDL-C levels were however found to be higher among the case participants than their control counterparts. This result agrees with the findings from previous studies in the Ghanaian and Nigerian populations (Nyarko *et al.*, 1997; Agrawal *et al.*, 2014). In the past HDL-C was thought to be cardioprotective, with efforts made to increase HDL-C levels to positively affect patients with atherosclerotic heart disease and reduce the risk of those with increased risk of atherosclerosis (Acquah *et al.*, 2012). However, emerging evidence shows that higher HDL-C levels may not necessarily result in decreased risk of cardiovascular disease but

rather the functional quality of HDL-C should be of sconsideration (Rader, 2012). In diabetes, the functional quality of HDL-C has been found to be lower than that of healthy controls, as HDL-C of diabetics is unable to reversetransport cholesterol, has impaired anti-inflammatory and anti-oxidative properties and may even be proarteriogenic (Kastelein *et al.*, 2007).

The association of haemodynamic parameters with glycaemia, TC and LDL-C observed in this study were reported recently among diabetes mellitus patients attending the Tamale Teaching Hospital in Ghana (Titty, 2010). Carbohydrate and lipid metabolic disorders exist in majority of subjects with hypertension and type 2 diabetes. The most fundamental defect in these patients is resistance to cellular actions of insulin which appears to cause hyperinsulinemia, enhanced hepatic gluconeogenesis and glucose output, reduced suppression of lipolysis in adipose tissue leading to a high free fatty acid influx and increased hepatic very low density lipoprotein (VLDL) secretion causing hypertriglyceridemia and reduced plasma levels of high density lipoprotein (HDL) cholesterol (Avramoglu *et al.*, 2006).

The inflammatory markers assayed in this study were hs-CRP, IL-6, TNF-α, WBC and ESR. With the exception of TNF-α levels, the study recorded higher levels of inflammatory markers in the case subjects than the control. High levels of inflammatory markers found in type 2 diabetes and hypertensive subjects in the current study add to several existing studies which reported similar

findings. Hs-CRP, IL-6, TNF-α, WBC and ESR are markers of low-grade systemic inflammation (Lee and Liu, 2008; Magen *et al.*, 2008) whiles WBC and ESR measurements serve as proxy markers for nonspecific inflammation (Twig *et al.*, 2013). Quartile distributions of the various inflammatory markers were compared between case and control groups in the study population. The trends of population cluster in general recorded increasing numbers of patients from first to the fourth quartiles of inflammatory markers whilst the reverse was observed among the control group (figure1&2). This is similar to results obtained by Marques-Vidal *et al.* (2012) where in the CoLaus study, in which with the exception of TNF-α, non diabetic subjects showed significantly different quartile distribution of inflammatory markers from those with incident type 2 diabetes with increasing population clusters from the first to the fourth quartiles.

In this study, hs-CRP levels positively correlated with anthropometric variables and haemodynamic parameters among the case group. Though the proposed links between adiposity, inflammation and hypertension are largely speculative, reports indicate that low-grade inflammation measured by CRP levels could be a marker of highly active cytokine producing adipose tissue infiltrated by macrophages and leading to complement activation, release of vasoactive cytokines and subsequent endothelial dysfunction (Weisberg *et al.*, 2003; Wellen and Hotamisligil, 2003). Endothelial dysfunction is known to

cause abnormal response to acetylcholine in hypertensive vessels, accounting at least in part for the increased vascular resistance observed in hypertension (Panza, 1997).

Control participants recorded significant association between glycaemic levels and IL-6 whiles in the patients the association was between glycaemic levels with hs-CRP. Previous studies have shown different results regarding the association between glycaemia and hs-CRP with Ford (1999), Aronson *et al.* (2004), Liu *et al.* (2002) and Marques-Vidal *et al.* (2012) recording positive association whiles (Rytter *et al.*, 2009) found no association between glycaemic control and inflammation among type 2 diabetic subjects. Various mechanisms have been indicated by which cytokines contribute to the development of type 2 diabetes (Pickup, 2004). Cytokines can directly inhibit insulin receptor signaling by activating c-Jun amino-terminal kinase and an inhibitor of nuclear factor kappa-beta kinase, leading to serine phosphorylation of insulin receptor substrate (Hotamisligil, 2006). In addition, these cytokines have been shown to promote hepatic fatty acid syntheses and induce the liver to produce more acute-phase proteins, as well as recruit more inflammatory cells to adipose tissue and pancreatic beta-cells (Butler *et al.*, 2003). Inflammatory cytokines do not only affect insulin resistance but may also contribute directly to beta-cell apoptosis and beta-cell failure, ultimately leading to type 2 diabetes (Lee and Liu, 2008).

The study also sought to determine the relative risk of developing future cardiovascular event among the different groups within the study population using the Framingham Risk Score (Brindle *et al.*, 2003). The general study population recorded 25% of subjects as having high risk of coronary heart disease over the next ten years. A further 25% recorded a moderate risk over the next ten years. Significantly all the subjects classified as having high risk of developing coronary heart disease were of the case group (39.1%) (Figure 1A). In the STARNet study, Parchman *et al.* (2007) found 16.2% of type 2 diabetic subjects recording a high risk of coronary heart disease in ten years. The observed difference in the percentage ten year coronary risk between the current study and that of the STARNet could be explained by the difference in population characteristics. Whereas the case participants in this study included type 2 diabetes and hypertensive subjects, the STARNet study consisted of only persons presenting with type 2 diabetes. Moreover, the high coronary risk observed among the case group in this study could be attributable to the major unfavourable cardiometabolic risk profiles exhibited by these individuals compared to the controls (Grundy *et al.*, 1999b). The presence of these classic and putative risk factors may affect the heart (coronary artery disease), the central nervous system (cerebrovascular disease) and the lower limbs (peripheral vascular disease) (Grundy *et al.*, 1999b).

Gender variations in percentage ten year risk estimations were observed in this study, with significantly greater proportion of female participants ($p<0.0030$) than male exhibiting high risk profile (Figure 3B & D). The result compares with those obtained in a meta-analysis of 37 prospective cohort studies which recorded a significantly higher ($P< 0.0001$) risk for fatal coronary heart disease associated with diabetes in women than in men (Huxley *et al.*, 2006). In explaining this phenomenon, Huxley *et al.* (2006) posited that women may present with more unfavourable cardiovascular risk profiles than men. Thus, women with diabetes have significantly higher levels of blood pressure and lipids than men with diabetes (Huxley *et al.*, 2006).

CHAPTER SIX
CONCLUSIONS AND RECOMMENDATIONS

Low-grade systemic inflammation plays a role in the pathogenesis of both insulin resistance and endothelial dysfunction and subsequently the development of type 2 diabetes and atherosclerosis.

The findings of this study is in tandem with reports from prospective studies of diverse populations that support a role of inflammatory cytokines in the clinical co-expression of type 2 diabetes and hypertension. In this study, inflammation was found to be associated with Ghanaians presenting with hypertension and type 2 diabetes undergoing medical care at the Battor Catholic Hospital. With the exception of TNF-α levels, participants with hypertension and type 2 diabetes were found to exhibit higher concentrations of hs-CRP, IL-6, WBC and ESR compared to their control counterparts.

Twenty-five percent of the study population recorded high risk of coronary heart disease over the next ten years. This could be attributable to the unfavourable cardiometabolic risk profiles recorded in these subjects compared to controls. Gender variations in percentage ten year risk of coronary heart disease was revealed as a significantly proportion of women had higher risk than men. Hence it is important that such persons are identified and managed properly so as to delay or prevent any future adverse outcome.

Although the results of this study support the use of the hs-CRP assay to identify high-risk patients who may benefit from primary prevention, they are also consistent with a putative causal role of hs-CRP in both type 2 diabetes and CHD events. High levels of hs-CRP may provoke vascular inflammation (Pasceri *et al.*, 2000), and preferentially bind to oxidized LDL (Chang *et al.*, 2002). Statins lower LDL-C level and LDL particle number, reduce hs-CRP level (Ridker *et al.*, 2001), and reduce Lp-PLA2 activity (Tsimihodimos *et al.*, 2002). However, many patients continue to have elevated hs-CRP even on statin therapy (Ballantyne *et al.*, 2004). Other therapies, such as weight loss and highdose aspirin, also reduce hs-CRP levels. Therefore, in addition to potentially identifying high-risk but currently untreated patients who may benefit from therapies such as statins to reduce CHD events, measurement of hs-CRP may be useful to identify cohorts of patients for clinical trials to determine whether reduction/inhibition of hs-CRP reduces CHD events.

In summary, hs-CRP may be complementary in identifying individuals with high CHD risk but low LDL-C (Ballantyne *et al.*, 2004).

The following are recommendations for further studies in future:

- Further works could expand the scope of biomarkers of inflammation and endothelial dysfunction to include interleukin 18, fibrinogen,

Eselectin, intercellular adhesion molecule-1 and vascular adhesion molecule-1 etc.

- Further studies could also compare oxidised LDL-Cholesterol levels between type 2 diabetes and hypertensive individuals with their healthy counterparts in evaluating their future cardiovascular risk.

- The sample size could be increased substantially so that a generalised conclusion could be made among the Ghanaian population.

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