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KUMASI

OXIDATIVE STRESS AND INFLAMMATION IN PATIENTS PRESENTING WITH HYPERTENSION AND TYPE 2 DIABETES AT THE SHAI-OSUDOKU DISTRICT HOSPITAL, DODOWA

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By

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DECLARATION

The experimental work described in this thesis was carried out at the Department of Molecular Medicine, KNUST. This work has not been submitted for any other degree.

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ABSTRACT

Increasing evidence has highlighted the roles of oxidative stress and inflammation in the promotion of hypertensive and type 2 diabetic complications. Inflammation which can lead to overproduction of reactive oxygen species triggers oxidative stress in patients presenting with hypertension and type 2 diabetes (T2DM). The purpose of this study was to evaluate oxidative stress and inflammatory markers in patients presenting with hypertension (HPTN) and T2DM. The study was conducted from October 2013 to July 2015 at the Diabetic/hypertensive clinic and Laboratory Unit of the Shai-Osudoku District Hospital, in the Shai-Osudoku District of the Greater Accra Region of Ghana. The study population consisted of 250 participants: 100 controls and 150 patients with T2DM, HPTN and HPTN/T2DM as "case groups". Based on clinical and laboratory criteria, the participants were classified into four (4) groups: normal participants (control=100), normotensive patients with T2DM (T2DM, n=50), patients with hypertension (HPTN, n=49) and hypertensive patients with T2DM (HPTN + T2DM, n=51.Serum Total Antioxidant Capacity was significantly decreased among case participants as compared to the controls (p=0.033). This decrease was more prominent (p-value < 0.01) in participants presenting with both chronic conditions (T2DM and HPTN) with mean value of 1.07±0.43Mm. Of all the inflammatory markers compared, case participants had higher levels, with significant differences in Erythrocyte Sedimentation Rate, ($p < 0.0001$) and the high-sensitivity C-reactive protein, (p=0.0011) but not Tumour necrosis factor-alpha, (P=0.883) and White blood cell, (p=0.1536). High-sensitivity C-reactive protein was significantly increased in T2DM ($p < 0.01$) and hypertensive participants ($p < 0.01$). The high levels were marked ($p < 0.001$) in participants presenting with T2DM/HPTN. The prevalence of metabolic

syndrome among the case participants was significantly higher than the control participants (21.3% vs. 2%) using National Cholesterol Education Programme, (NCEP ATP III) criteria. Intra-group metabolic syndrome (MetS) differences was observed among the cases, with the proportion of MetS increasing from those presenting with HPTN (8.2%) and with T2DM (25.5%) to a maximum among the participants presenting with T2DM/HPTN (30%). Lipid indices except high density lipoprotein cholesterol were found to be higher among the case participants than the control participants ($p < 0.0001$ each)**.** Patients with the two associated diseases have a more active inflammatory and oxidative stress. MetS has been demonstrated to be significantly associated with inflammation in patients with HPTN and T2DM. Both T2DM and HPTN are strongly associated with hyperlipidaemia and obesity, besides being powerful risk factors for cardiovascular disease. Since inflammation is known to affect T2DM and HPTN in pathways that implicate endothelial dysfunction, hence endothelial markers of vascular integrity such as VCAM-1, Oxidized LDL and Nitric Oxide in the various disease groups can be investigated.

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ABBREVIATIONS

ACE.....Angiotensin-converting enzyme

ADA- .....American Diabetes Association

ADP.....Adenosine dinucleotide phosphate-ribose

AGE.....Advanced glycation end product

ARE-.....Antioxidant response elements

ATP III.....Adult Treatment Panel III

BMI- .....Body Mass Index

CAD .....Coronary artery disease

COX 2- cyclo-oxygenase-2

CVD.....Cardiovascular disease

DAG- .....diacylglycerol

EDTA- .....Ethylene diamine tetra acetic acid

ESH-ESC-…...European Society of Hypertension and European Society of Cardiology

GAPDH.....Glyceraldehydes-3 phosphate dehydrogenase (GAPDH)

GFAT.....Glutamine fructose-6-phosphate amidotransferase

GPx.....Glutathione peroxidase

GSH.....Reduced to oxidised glutathione

HPTN……Hypertension

Hs-CRP.....High sensitivity C- reactive protein

IDF......International Diabetes Federation

IL-6.....Interleukin 6

IRS-.....Insulin resistance syndrome

ISH.....International Society of Hypertension

JNK-c-.....Jun N-terminal kinase

KATH- .....Komfo Anokye Teaching Hospital

MCP-1.....monocyte chemoattractant protein 1

MMP-9- .....Matrix metalloproteinase-9

NADPH......Nicotinamide-adenine dinucleotide phosphate

NCEP.....National Cholesterol Education Programme

NIH- .....National Institute of Health

PAI-1..... ….Plasminogen activator inhibitor type 1

PARP.....adenosine dinucleotide phosphate-ribose

PPAR-.....Peroxisome-proliferator-activated receptor

SCORE.....European Systematic Coronary Risk Evaluation

SOD......Superoxide dismutase

T2DM.....Type 2 Diabetes Mellitus

TAC- .....Total antioxidant capacity

Val-MARC... Valsantan-Managing blood pressure aggressively and evaluating

VAT.....Visceral adipose tissue

VSMCs......Vascular smooth muscle cell

WC-.....Waist circumference

WHR-.....Waist-hip-ratio

Chapter 1

INTRODUCTION

1.1 GERERAL INTRODUCTION

Cardiovascular disease remains the leading cause of death worldwide, though over the last two decades, cardiovascular mortality rates have declined in many high-income countries but have increased at an astonishingly fast rate in low-and middle income countries. The percentage of premature deaths from cardiovascular disease range from 4% in high-income countries to 42% in low-income countries. More than 17 million people died from cardiovascular disease in 2008 (Mendis *et al.*, 2011). Worldwide, HPTN is common and now regarded as a major public health problem (Murray and Lopez, 1997). HPTN is now being widely reported in Africa and is the most common cause of cardiovascular disease on the continent (Cooper and Rotimi, 1992). In Ghana, earlier studies revealed a HPTN prevalence of 4.5% among rural dwellers and of 8% to 13% in the town (Pobee, 1992). The prevalence of HPTN in Ghana (BP ≥140/90 mmHg with or without antihypertensive treatment) ranged from 19% to 48% between studies (Bosu, 2010). 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. The prevalence of diabetes is higher in men than women, but there are more women with diabetes than men (Sarah *et al.,* 2004). 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 (Dodu and De Heer, 1964). Diabetes screening conducted by the Ghana Diabetes Association in the early 1990s suggested 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 was recorded in a community in Accra (Amoah *et al.*, 2002). The frequency of diabetes mellitus is increasing rapidly worldwide even in developing countries (Zimmet *et al.*, 2001). HPTN often co-exists with diabetes, such that 60% of patients with diabetes are hypertensive, and up to 20% of subjects with HPTN are diabetic (Contreras *et al.*, 2000). In patients with T2DM and in HPTN, the pathophysiology of cardiovascular disease is multifactorial, and endothelial dysfunction and vascular inflammation develop with time and are independently associated with mortality (Stehouwer *et al.*, 2002). There is compelling evidence that oxidative stress, inflammation, HPTN and T2DM are involved in a self-perpetuating vicious cycle which, if not interrupted, culminates in progressive target organ injury and dysfunction. Inflammatory markers and oxidative stress are increased in patients with HPTN and metabolic disorders, and predict the development of cardiovascular disease (Savoia and Schiffrin, 2006). Thus, in the presence of oxidative stress, ability of the endothelial cells to maintain vascular homeostasis is diminished and the endothelium can become infiltrated by lipids and leucocytes which initiates an inflammatory response and produces the initial atherosclerotic lesion of a fatty streak (Okoduwa *et al.*, 2013). Oxidative stress (OS), through the production of reactive oxygen species (ROS), has been proposed as the root cause underlying the development of insulin resistance, beta-cell dysfunction, impaired glucose tolerance ,T2DM and HPTN, it has also been implicated in the progression of long-term diabetes complications (Moussa, 2008). Excess nourishment and a sedentary lifestyle leads to glucose and fatty acid overload, resulting in production of ROS. Additionally, reaction of glucose with plasma proteins forms advanced glycation end products (AGEs),

triggering production of reactive oxygen species (ROS). These ROS initiate a chain reaction leading to reduced nitric oxide (NO) availability, increased markers of inflammation and chemical modification of lipoproteins, all of which may increase the risk of atherogenesis and diabetic and hypertensive complications (Wright *et al.*, 2006; Garcia-Bailo *et al.*, 2011). There is strong evidence that inflammation forms the basis in the pathophysiology of both insulin resistance and CVD among type 2 diabetic (T2DM) patients (Sobel and Schneider, 2005). CVD has several underlying risk factors including the conventional HPTN, hyperglycemia, increased levels of total cholesterol and low density cholesterol (LDL) and several other factors like race, origin, gender and smoking, lack of exercise, DM, genetics (Hamdy *et al.*, 2006) as well as the non-conventional risk factors like considerable variations in the levels of inflammatory markers including C-reactive protein (CRP), Interleukine-6 (IL-6), Tumour necrosis factor (TNF-α), C-peptide and fatty acid binding protein (FABP) are all involved in the pathogenesis of insulin resistance (D'Souza *et al.*, 2009). Hyperglycemia is known to stimulate the release of inflammatory cytokines from various cell types and can lead to the induction and secretion of acute-phase reactants by adipocytes. The serum level of high sensitivity C-reactive protein (hs-CRP), which is a marker of systemic inflammation and a mediator of atherosclerotic disease, have been correlated with the risk of cardiovascular disease and T2DM (Kang *et al.*, 2005; Akash *et al.*, 2013). Such oxidative stress and subclinical inflammatory process can be characterized by elevated circulating levels of Total antioxidant capacity, high sensitivity C-reactive protein and Tumor Necrosis Factor-α (Madamanchi *et al.*, 2005). Metabolic syndrome is known to predispose to atherosclerosis. High sensitivity-C-reactive protein (hs-CRP), a marker of

systemic inflammation is significantly associated with the components of metabolic syndrome. Both obesity and metabolic syndrome have been independently linked with increased inflammatory stress in patients presenting with HPTN and T2DM (Vidyasagar *et al.*, 2013).

1.2 JUSTIFICATION

Cardiovascular diseases (CVD) have become a leading cause of mortality and morbidity in developing countries and rates are expected to rise further over the next few decades (Yusuf *et al.*, 2001; Sarah *et al.*, 2004; Kearney *et al.*, 2005). Trends have changed in recent years. There are epidemiological and demographic transitions taking place in developing countries with declining rates of communicable diseases and increasing rates of non-communicable diseases (Erdine and Aran, 2003). The life expectancy of people in developing countries has increased. There are significant changes in the lifestyle and socio-economic status of people. People in developing countries are adopting a western lifestyle. Urbanization, industrialization and ageing of the population are growing very rapidly in developing countries; all play an important role behind the increasing prevalence of HPTN and T2DM. However there is scarcity of health resources and infrastructure, which leads to an inadequate control of HPTN and T2DM (Erdine and Aran, 2003). Over the last few years, there have been a lot of promising clinical markers proposed to link oxidative stress, inflammation, HPTN and T2DM (Vaziri and Rodríguez-Iturbe, 2006). Measuring oxidative stress and inflammatory markers in serum may provide clinicians with additional information regarding patient's risk of developing cardiovascular disease (Vaziri and Rodríguez-

Iturbe, 2006). Overwhelming evidence has accumulated indicating that oxidative stress markers and inflammatory cytokines play a role in the pathogenesis of T2DM and HPTN. Measuring oxidative stress and inflammation may increase cardiovascular disease risk predictions (Savoia and Schiffrin, 2006). The increasing burden of CVD has important economic implications. CVD occurs typically at a younger age in developing than developed countries with important consequences such as loss of revenue at household level and loss of productivity at a macroeconomic level. From a health system perspective, huge resources are needed for providing health care to large numbers of chronic patients for decades and for sustaining increasingly sophisticated equipment and more skilled and harder-to-replace workforce (Beaglehole & Yach, 2003). In order to design effective messages for public education and good interventions there is the need to clearly define the role of oxidative stress and inflammation in patients presenting with HPTN and T2DM. However there is paucity of data on oxidative stress and inflammatory markers in T2DM and hypertensive patients in the Sub-Saharan Africa. In addition, studies conducted earlier shows that there is inconsistency in the levels of inflammatory and oxidative stress markers associated with T2DM and HPTN. Despite the roles these oxidative stress and inflammatory markers play in HPTN and T2DM, there is little or virtually no laboratory-supported information available to show that clinicians request for these markers in the assessment of HPTN and T2DM in clients who call at their facilities in Ghana. The outcome of this study will help clinicians in making decisions about the assessment of this condition in response to therapeutic lifestyle interventions. It will also help policy makers to design interventions to improve the health of our people.

1.3 GENERAL OBJECTIVE

The purpose of this study was to evaluate oxidative stress and inflammatory markers among patients presenting with HPTN and T2DM.

1.4 SPECIFIC OBJECTIVES

- To evaluate oxidative stress among patients presenting with HPTN and T2DM.
- To identify inflammatory markers (Hs-CRP, TNF-α, ESR) associated with HPTN and T2DM.
- To determine the relationship between components of metabolic syndrome using (NCEP ATP III) Criteria and inflammation among patients presenting with HPTN and T2DM.
- To determine the differences in anthropometry and lipid parameters among the study participants.

Chapter 2

LITERATURE REVIEW

2.1 DIABETES MELLITUS

Diabetes mellitus is a metabolic disease which, when not properly treated or untreated is characterized by chronic hyperglycaemia and disordered carbohydrate, lipid and protein metabolism and is associated with the development of specific microvascular complications and of non-specific macrovascular disease (Aalto, 1999). From the insulin angle of reasoning, diabetes mellitus is described as a group of chronic metabolic conditions, all of which are characterized by elevated blood glucose levels which is resulted from the inability of the body to produce insulin or resistance to insulin action, or both (Deshpande *et al.*, 2008). It has been described as a killer disease in so many situations. Diabetes is now ranked among one of the most common non-communicable diseases in the world. It falls within 4th–5th leading cause of death in most developed countries and there are facts and figures that it is epidermic in many developing and newly industrialized countries. Diabetes is a progressive condition initially characterized by insulin resistance, where muscle and adipose tissue become relatively insensitive to the effects of insulin. As the condition progresses, declining beta cell activity results in relative insulin deficiency and blood glucose levels rise above normal levels (Hill, 2009). Meanwhile, the global burden of diabetes was 110 million in mid 90's and it is projected to increase to 221 million by the year 2010 (Park, 2004).

2.1.1 Type 1 DM

This type accounts for 10% of all cases of DM (Cotran *et al.*, 1999). In this type a T cell mediated autoimmune disease destroys pancreatic β-cells and causes rapid loss of

insulin secretory reserves. This cell autoimmune destruction commences in the first 5 years of life until symptoms of type 1 DM appear clinically; at that time most of the β-cells (80%) has already been destroyed. This disadvantage led the scientist to focus on preventing the disease by identifying patient in their preclinical phase of the disease (Okoduwa *et al.*, 2013) using several immunological markers, reflecting ongoing immune activity and possible β-cell destruction.

2.1.2 Type 2 DM

This type accounts for 80-90% of cases of DM (Cotran *et al.*, 1999). It is generally characterized by relative insulin deficiency and peripheral tissue insulin resistance (Khanna *et al.*, 2008). Type 2 DM has a strong genetic predispotion (Cotran *et al.*, 1999),as it appears to result from a collection of multiple genetic defects or polymorphisms (Cotran *et al.*, 1999). Life style plays a major role as obesity is common in people with T2DM and it is known that obesity itself causes insulin resistance (Khanna *et al.*, 2008).

2.1.2.1 Insulin Deficiency:

The cause of insulin deficiency is not clear but strong evidence shows irreversible β-cell damages. According to a certain view, all somatic cells of predisposed individuals including β-cells are genetically vulnerable to injury leading to accelerated cell turn over and premature aging. Also chronic hyperglycemia may exhaust the ability of β-cell to function (glucose toxicity) (Cotran *et al.*, 1999).

2.1.2.2 Insulin Resistance:

Insulin resistance is defined as a decreased biological response to normal concentrations of circulating insulin and is found in both obese, nondiabetic individuals and patients with T2DM (Tietz, 2006). Insulin resistance is manifested by a decrease in the ability of

skeletal muscle to store glucose (due to decrease in the activity of glycogen synthase) and to oxidize glucose (due to decrease activity of pyruvate dehydrogenase) (Khanna *et al.*, 2008). Also the hepatic cells show an increase in glucose output due to inhibited glycolysis and increased gluconeogenesis which lead to chronic hyperglycemia (Khanna *et al.*, 2008).

2.1.2.3 Family History

Type 2 Diabetes is more common in individuals with a family history of the disease (Cotran *et al.*, 1999) or cardiovascular disease (hypertension or dyslipidemia) (Khanna *et al.*, 2008). Family history of T2DM (genetic predisposition), the duration of obesity, and the distribution of fat are all considered important factors affecting the incidence of T2DM.

2.1.2.4 Amylin

Amylin (also called islet amyloid polypeptide [IAPP]) is a 37 amino acid peptide produced by the β-cells co-packaged with insulin and co-secreated with it in response to food ingestion. In type 2 diabetic patients amylin tends to accumulate in the sinusoidal space outside the β-cells, until it acquires the peculiar characteristic of amyloid (Chan *et al.*, 1994). But it is still unknown if this contributes to the disturbance in glucose sensing by the β-cells (Cotran *et al.*, 1999). It is also a matter of controversy, whether amylin deposition contributes to the pathogenesis or is a consequence of T2DM. Although this is an area of ongoing research, at the present time, there is no clinical value in measuring amylin (Tietz, 2006).

2.2 Oxidative stress

Oxidative stress is characterized by persistent imbalance between excessive production of reactive oxygen species (ROS) and/or reactive nitrogen species (Naito *et al.*, 2010) and limited antioxidant defenses. ROS are either charged species, such as superoxide (O2 -•), precursor of ROS, and hydroxyl radical (OH•); or uncharged species, *i.e.* hydrogen peroxide (H_2O_2). Superoxide can be converted to less reactive H_2O_2 by superoxide dismutase (Sandhu *et al.*, 2005) isoenzymes. H_2O_2 can in turn be degraded to O_2 plus H_2O by catalase (CAT), glutathione peroxidase (GPx), and peroxiredoxin, enzymes which constitute antioxidant defenses (Rhee *et al.*, 2005). ROS formation is an inherent and essential phenomenon of life and small fluctuations in the steady-state concentrations of oxidants may contribute to physiological control of cell functions (Castro and Freeman, 2001; Rhee *et al.*, 2005). However, uncontrolled increase of oxidants, or reduction of their detoxification, leads to free radical-mediated chain reactions ultimately targeting cellular proteins, lipids, polysaccharides, and DNA (Imlay *et al.*, 1988). This might initiate a pathogenic cascade of events (Evans *et al.*, 2002).

2.2.1 Oxidative stress and diabetes

2.2.1.1 Increased oxidative stress in diabetes

Increased oxidative stress and free radical-induced damages have been proposed to be implicated in the diabetic state (Yu, 1994). In type 1 diabetes, ROS participate in ß-cell dysfunction initiated by autoimmune reactions and inflammatory cytokines (Rabinovitch, 1998). In T2DM, excessive ROS impair insulin synthesis (Evans *et al.*, 2002) and activate ß-cell apoptotic pathways (Mandrup-Poulsen, 2001; Evans *et al.*, 2002). Hyperglycemia induces generation of superoxide at the mitochondrial level in endothelial cells and triggers a vicious cycle of oxidative stress in the development of

diabetic complications (Nishikawa *et al.,* 2000). In the rat Zucker diabetic fatty model of T2DM, direct measurements of superoxide in isolated pancreatic islets revealed ROS generation coupled to mitochondrial metabolism and perturbed mitochondrial function (Bindokas *et al.*, 2003). Alloxan induced insulin-dependent diabetic animal models provide further evidence supporting the involvement of free radicals in diabetogenesis. Following its selective uptake by ß-cells through GLUT2 glucose transporter, alloxan generates ROS in a cyclic redox reaction along with its reduction product dial uric acid. This leads to selective necrosis of ß-cells (Badawi *et al.*, 2010). Supplementation of free radical scavengers, such as SOD and vitamin E, efficiently removes oxidants and protects against the diabetogenic action of alloxan (Donath *et al.*, 2008; King, 2008).

2.2.1.2 Source of ROS in diabetes

Several conditions leading to ROS generation in diabetes have been proposed, among them gluco-lipotoxicity and mitochondrial pathways.

2.2.1.3 Lipids

In addition to hyperglycemia, the type 2 diabetic state is also characterized by increased fasting and postprandial plasma levels of triglycerides and free fatty acids, molecules known to favor ROS generation (Inoguchi *et al.*, 2000). Specifically, excessive levels of palmitate are associated with abnormal islet functions that lead to increased lipid esterification, in turn producing ceramides that induce oxidative stress (Briaud *et al.*, 2001). Noteworthy, antioxidant therapies have been proposed to protect ß-cells from gluco-lipotoxicity. For instance, metformin and troglitatone, both of which exhibiting antioxidant properties, can prevent hyperglycemia in the Zucker diabetic fatty (ZDF) rat (Harmon *et al.,* 1999). Troglitazone also prevents increased levels of lipid hydroperoxide in another rat model of T2DM (Fukui *et al.*, 2000).

2.2.1.4 Hyperglycemia

Oxidative stress plays a key role in the pathogenesis of vascular complications and in T2DM. During chronic hyperglycaemia, β cells are exposed to high glucose concentration for an extended period of time. Hyperglycaemia-induced oxidative stress directly promotes endothelial dysfunction via several mechanisms including glucose auto-oxidation, the formation of advanced glycation end products (AGEs) and activation of the polyol pathway (Jay *et al.*, 2006).

2.2.1.4.1 Glucose Auto-Oxidation

Recently, a unified mechanism has been proposed by (Brownlee, 2005) to explain the role of hyperglycaemia-induced oxidative stress in diabetic complications. Upon hyperglycaemia, there is an increase in electrons entering the mitochondrial electron transport chain during glucose oxidation. The voltage gradient across the mitochondrial membrane will therefore rise beyond a certain threshold so that the electron transfer is blocked inside complex III of the electron transport chain. Electron leaking then occurs at the position previous to complex III (i.e. Coenzyme Q) to generate superoxide. Consequently, over production of superoxide from the mitochondrial electron transport chain inhibits the activity of the key glycolytic enzyme glyceraldehydes-3 phosphate dehydrogenase (GAPDH) by the following steps;

- Causing strand breaks in nuclear DNA and activating poly (adenosine dinucleotide phosphate-ribose) polymerase(PARP),
- Synthesising adenosine dinucleotide phosphate-ribose (ADP-ribose) polymers with PARP, and
- Binding of ADP-ribose to GAPDH.The inhibition of GAPDH will trigger four major mechanisms of hyperglycaemia-induced damage: the polyol pathway,

the hexosamine pathway, the protein kinase C pathway and the advanced glycation end products pathway, which contributes to the development of diabetic complications.(Brownlee, 2005).

Hyperglycaemia is reported to deplete antioxidants and to increase oxidative stress, and this may be central to the development of vascular complications in diabetes (Laight *et al.*, 2000). Increased oxidative stress is suggested as a major contributor to insulin resistance and associated accelerated atherosclerosis, and possibly to diabetic complications including renal damage, retinopathy and neuropathy (Laight *et al.*, 2000).

2.2.1.4.2 Polyol Pathway:

Some tissues do not require insulin for glucose transport (such as nerves, lens, kidneys and blood vessels). Therefore, large amounts of glucose may enter the cells of these tissues/organs during times of hyperglycemia, leading to increase intracellular glucose (Cotran *et al.*, 1999). The polyol pathway in these tissues becomes active with the increased intracellular glucose level (Oates, 2002). The pathway is a two step metabolic pathway in which glucose is reduced to sorbitol (Lorenzi, 2007) in a reaction catalyzed by the enzyme aldose reductase (AR) (which is the first rate limiting enzyme in the pathway) and it uses NADPH as a cofactor. Sorbitol is then oxidized to fructose by sorbitol dehydrogenase (SD) that uses NAD+ as a cofactor, and is present in cells of the liver, ovaries, sperms, and seminal vesicles (Lorenzi, 2007). Elevated intracellular glucose concentrations and an adequate supply of NADPH cause AR to produce a significant increase in the amount of sorbitol, which cannot pass efficiently through cell membranes and, therefore, remains trapped inside the cell. This is exacerbated when SD is low or absent, for example, in retina, lens, kidney, and nerve cells. As a result, sorbitol accumulates in these cells, causing strong osmotic effects and, therefore,

increased influx of water and cell swelling eventually leading to osmotic cell injury associated with diabetes. Some of the diabetes complications can be attributed, at least in part, to this phenomenon, including cataract formation, peripheral neuropathy, and vascular problems leading to nephropathy and retinopathy (Cotran *et al.*, 1999). In addition, the fructose produced by this pathway can be phosphorylated to fructose-3-phosphate (Cotran *et al.*, 1999) which is broken down to 3 deoxy glucosone which are both considered powerful glycosylating agents, entering in the formation of Advanced Glycosylating Agents (AGEs) (Szwergold *et al.*, 1990). Furthermore, due to the usage of NADPH by aldose reductase, less cofactor becomes available for glutathione reductase which is critical for the maintenance of the intracellular pool of reduced glutathione (GSH). This will lessen the capability of cell to respond to oxidative stress (Barnett *et al.*, 1986). Thus activation of the polyol pathway by altering the intracellular osmolarity generates AGEs precursors and exposes cells to oxidative stress. This may occur through decreased antioxidant defenses and generating oxidant species that can initiate multiple mechanisms of cellular damages (Lorenzi, 2007). The previous biochemical explanations have been considered as causative factors in the development of cataract, retinal damage, peripheral neuropathy and early functional derangements in the kidney (Cotran *et al.*, 1999).

2.2.1.4.3 Non enzymatic glycosylation:

Glucose without the aid of an enzyme chemically condenses with amino acids of cellular proteins, which forms a reversible glycosylation product with Schiff bases and this product may rearrange to form a more stable product called Amadori early-glycated products. The glycosylation product on collagen and other long lived proteins in interstitial tissues and blood vessels walls do not dissociate and undergoes a series of

chemical rearrangements, dehydration, and fragmentation reactions, forming irreversible stable advanced glycation end products (AGE) (Cotran *et al.*, 1999). The amount of these end products do not return to normal when hyperglycemia is corrected, and they accumulate continuously over the lifespan of the protein. Thus the function of the protein is altered or inactivated, and it is through these functional derangements that AGEs may contribute to the microvascular and macrovascular complications of DM. AGEs affect many cells in several ways, they can bind to receptors such as on monocytes (causing monocytes emigration), macrophages (causing release of cytokines and growth factors), lymphocytes, endothelial cells and other cells (increase permeability, increase procoagulant activity and enhanced proliferation and synthesis of extracellular matrix by fibroblasts and smooth muscle cells) (Cotran *et al.*, 1999), all these effects contribute to diabetic complications.

2.2.1.4.4 Protein Kinase C (PKC):

Hyperglycemia induces de novo diacylglycerol synthesis which activates protein kinase C (PKC) (Lee *et al.*, 1989), which has been implicated in many processes related to Diabetic complications such as cardiovascular complications (Giardino and Brownlee, 1997). In diabetes and insulin resistance, activation of PKC in vascular cells may be a key link between elevated plasma and tissue concentrations of glucose and free fatty acids and abnormal vascular cell signaling (Rask-Madsen and King, 2005).

2.2.1.4.5 Hexosamine pathway:

O-linked glycosylation of certain proteins is increased in DM because cellular uptake of glucose activates the hexosamine pathway which results in production of uridine diphosphate-N-acetyl-glucosamine, a substrate in the glycosylation reaction. Furthermore, in diabetics the expression of glutamine: fructose-6-phosphate

amidotransferase (GFAT), the rate limiting enzyme for this pathway, is increased in several tissues prone to complications. Through activation of this pathway, hyperglycemia may lead to O-linked glycosylation of endothelial nitric oxide synthase (eNOS) on serine 1177 prohibiting activation of the enzyme by phosphorylation on this residue. In vitro studies in mesengial cells have demonstrated that high levels of glucose, glucosamine or GFAT increased the O-linked glycosylation of p65 NF-B; a nuclear factor which activates several gene programs among the pro-inflammatory genes (Grundy *et al.*, 2005).

2.3. Oxidative Stress Biomarkers:

The increasing interest in the role of free radicals in the pathogenesis of human disease has led to widespread attempts to develop techniques suitable to measure free radicals and their reactions *in vivo*, specifically, in clinical pathology (Palmieri and Sblendorio, 2007). Since ROS/RNS themselves are very reactive and have an extremely short half life, their direct determination in tissue or body fluid are impracticable, therefore measurement of oxidativly/nitrosativly modified DNA, protein, lipids and sugars are appropriate biomarker for diseases in which ROS/RNS are involved (Shoelson *et al.*, 2006; Ogino and Wang, 2007; Zhang *et al.*, 2009**).**

2.3.1 Lipid Peroxidation Biomarkers:

Lipid peroxidation is a complex process and a wide range of products are formed in variable amounts. The major products are: (Zhang *et al.*, 2009)

1) α, β-unsaturated reactive aldehydes including:

4-hydroxy-2-nonenal (HNE) and Malondialdehyde (MDA)

2) propenal (acrolein) including Isoprostanes

2.3.2 Protein Oxidation Biomarkers:

Oxidative damage is induced either directly or indirectly: by reaction of secondary products leading to peptide backbone cleavage or fragmentation, cross-linking, altered susceptibility to proteolytic enzymes. Most damage is irreparable and may have a wide range of downstream consequences affecting the function of receptors, enzymes, transport proteins etc, and may generate new antigens provoking an immune response (Pitocco *et al.*, 2010).

2.3.2.1 DNA Modification:

Free radicals induce several types of DNA damage including: (Naito *et al.*, 2010).

a- Strand breaks.

b- DNA-protein cross-links.

c- Large range of base and sugar modifications.

The most representative biomarker of DNA oxidative damage in the cell is 8-hydroxy -2`- deoxyguanosine. Among a multitude of oxidants that can be measured in vitro and in animal models, only comparatively few biomarkers have entered epidemiological investigations like homocysteine, nitrosated tyrosines, and the relatively unstable F2-isoprostanes, which have been related to endothelial function or cardiovascular outcome in cross-sectional and prospective investigations (Naito *et al.*, 2010). As an alternative approach, investigators have tried to assess the capacity of antioxidant defense. Concentrations of glutathione, especially the ratio to oxidized glutathione, have been suggested as indicators of oxidative imbalance (Schnabel and Blankenberg, 2007).

2.4 Inflammation

Inflammation is defined as the local response to tissue injury. It is characterized by immune cell invasion and local release of cytokines and chemokines and is sometimes accompanied by functional or structural damage of the invaded tissue. It is not a disease, but a manifestation of disease. Inflammation has beneficial effects such as preventing spread of infections or promoting regeneration. Equally, it may exacerbate disease by tissue destruction due to inflammatory mediators, reactive oxygen species, and complement components (Donath *et al.*, 2008; Badawi *et al.*, 2010). Inflammation, which is currently considered to be a major factor in the development of T2DM, has also been proposed as a major factor in the development of diabetic neuropathy in animal models (Yagihashi *et al.*, 2011). In T2DM, inflammation and activation of monocytes are postulated to be important for enhancing insulin resistance and may contribute to the loss of insulin secretory function by islet cells, the above information is based on animal studies; however, information regarding the role of inflammation in human diabetic neueropathy is scarce (Doupis *et al.*, 2009).

2.4.1 Inflammatory Process, Insulin Resistance and Type 2 Diabetes

There has been a recent explosion of interest in the notion that chronic low grade inflammation and activation of the innate immune system are closely involved in the pathogenesis of T2DM (Wellen and Hotamisligil, 2005). It was demonstrated that markers of inflammation predict and / or are associated with T2DM and that inflammation is involved in the pathogenesis of atherosclerosis, a common complication of T2DM (Vîrgolici *et al.*, 2008). Insulin resistance begins prior to the onset of T2DM, at which time impaired glucose tolerance occurs as a result of beta cell decomposition and relative insulin deficiency (Association, 2010). Several factors are

linked to the development of insulin resistance in individuals with impaired glucose tolerance and T2DM, including genetics and environmental influences, obesity, and other conditions associated with chronic inflammation or infection (Huffman *et al.*, 2010). The possibility that obesity, and the activation of adipose tissue in particular, may enhance the release of inflammatory factors that underlie the development of insulin resistance has generated intense interest in the field of diabetes for a number of reasons (Gariballa *et al.*, 2013). First**,** a significant proportion of individuals with T2DM are overweight or obese, and obesity is a risk factor for the development of T2DM (King, 2008). Second, the increased release of adipocyte derived metabolites, such as fatty acids, and various inflammatory cytokines, in obese individuals has been linked to the development of insulin resistance (Shoelson *et al.*, 2006; Nishimura *et al.*, 2009). Third, chronic inflammation is associated with obesity, insulin resistance, and T2DM, all of which are features of the clustering of metabolic pathologies known as ‘‘metabolic syndrome (Hotamisligil, 2006).

2.4.2 Inflammatory Biomarkers in Type 2 Diabetes Mellitus

It is estimated that up to 25% of patients with newly diagnosed T2DM already present evidence of systemic inflammation at the time of diagnosis (Zhang *et al.*, 2009)**.**

2.4.2.1 **Tumor Necrosis Factor-alpha** (TNF-α)

TNF-α is a cytokine involved in systemic inflammation and is a member of a group of cytokines that stimulate the acute phase reaction. The major cellular source of TNF-α is activated mononuclear phagocytes, antigenstimulated T-cells, natural killer (NK) cells, and mast cells. The TNF-α gene is constitutively expressed in adipose tissue, where it originates principally from macrophage infiltration rather than from the adipocytes themselves (Hotamisligil *et al*., 1993). High TNF-α is related to the pathophysiology of

insulin resistance and T2DM. (Pickup, 2004) by enhancing the apoptosis of pancreatic β-cells (Pittas *et al.*, 2007). Moreover increased TNF-*α* production secondary to hyperglycemia may be a factor that exacerbates insulin resistance in poorly controlled diabetes because TNF-*α* induces insulin resistance (Fernández-Real and Ricart, 1999). The molecular mechanisms by which TNF-*α* induces insulin resistance are considered to be the following: TNF-*α* binds TNF receptor 1 and activates sphingomyelinase that metabolizes sphingomyelin to ceramide (Peraldi *et al.*, 1996), ceramide increases serine phosphorylation of insulin receptor substrate-1(IRS-1), which inhibits the insulin receptor tyrosine phosphorylation, resulting in attenuation of insulin signaling and a decrease in glucose transporter-4 (GLUT-4) translocation and glucose uptake. TNF-*α*–mediated insulin resistance associated with obesity may be related to metabolic syndrome (Satoh *et al.*, 2003; Liu *et al.*, 2006).

2.4.2.1.1 Tumor Necrosis Factor-alpha and Chronic Diabetic Complications

Diabetes is associated with excessive TNF-expression; this may result from constitutive overproduction by adipose tissue in T2DM, the effects of hyperglycemia and advanced glycation end products (Liu *et al.*, 2006). Chronic hyperglycemia activates macrophages and stimulates in vivo TNF-*α* production (Satoh *et al.*, 2003). Enhanced TNF-*α* production in a diabetic state may promote the development of diabetic micro- and macroangiopathies through a variety of TNF-*α* bioactivities. For example, TNF- *α* increases the permeability of the endothelium through release of nitric oxide (Ferro *et al.*, 1997) and it also increases thrombogenesis through plasminogen activator inhibitor-1 (PAI-1) over expression.. Furthermore, TNF-*α* stimulates the expression of adhesion molecules on the endothelial cells. The serum levels of free adhesion molecules significantly correlate with the intima-media complex thickness (IMT) of the carotid

artery. These imply that TNF-α accelerates atherosclerosis by inducing the expression of adhesion molecules on the endothelial cells (Satoh *et al.*, 2003).

2.4.2.2 Interleukins

Interleukins (IL) are a group of cytokines named for their ability to communicate between leukocytes. In recent years, research has shown their involvement in cell signaling in a number of other cell types and tissues. There are more than 30 interleukin isoforms currently identified that can have either pro-inflammatory (IL-1, IL-6 and IL-8) or anti-inflammatory (IL-2, IL-4 and IL-10) actions (Donath *et al.*, 2008). Interleukins have been shown to be involved in a number of different neuropathic conditions in both animal and human studies. Clinical studies in small-fiber neuropathy patients demonstrated a two-fold increase in circulating IL-2 mRNA levels in peripheral blood compared to healthy controls (Üçeyler *et al.*, 2010).

2.4.2.2.1 InterLeukin-6 (IL-6)

IL-6 is produced by many cell types including fibroblasts, endothelial cells, and monocytes-macrophages (Fain *et al.*, 2004). However, a significant proportion of the circulating IL-6 (15%–30%) is derived from adipose tissue production in the absence of acute inflammation to modulate adipocyte glucose and lipid metabolism (Orban *et al.*, 1999). IL-6 also triggers the hepatic synthesis of CRP and correlates with its serum levels, additionally, IL-6 promotes hepatic VLDL secretion and hypertriglyceridemia, these observations suggest a link between IL-6 levels, obesity and inflammation in the pathogenesis of T2DM and demonstrate that IL-6 can be considered as a candidate biomarker for early T2DM risk detection (Badawi *et al.*, 2010; Tetsuji, 2012).

2.4.2.3 C -reactive protein (CRP)

CRP is an acute-phase reactant produced primarily in the liver under the stimulation of adipocyte-derived IL-6 and TNF-α. It exhibits several characteristics that imply a fundamental immunoregulatory function, specifically; CRP is a member of the pentraxin family of oligomeric proteins which enhances leukocyte reactivity, complement fixation, modulation of platelet activation, and clearance of cellular debris from sites of active inflammation. The magnitude and rapidity of its induction and its cooperative role in innate immune response, as well as its ease of measurement, make CRP a common marker for inflammation (Mahajan *et al.*, 2009; Akash *et al.*, 2013). Furthermore, CRP is invariably correlated with various parameters relevant to diabetes, including obesity, lipogenesis, and adiponectin (Sattar *et al.*, 2008). In this respect, CRP was shown to be consistently associated with the incidence of T2DM in populations of otherwise healthy persons (Dehghan *et al.*, 2007). These findings substantiate a role for CRP as a possible candidate biomarker for T2DM (Hu *et al.*, 2004). A major mechanism by which CRP plays a critical role in T2DM is primarily by its action on pancreatic β-ell (Pfützner *et al.*, 2006). For example, CRP significantly inhibits cell proliferation and increases the rates of apoptotic cell death. This effect was connected to the CRP-mediated modulation of protein kinase B (PKB), a key factor for cell survival pathways and to its ability to induce the production of TNF-α, IL-1β and matrix metalloproteinase-9 (MMP-9) in a concentration dependent manner (Nabata *et al.*, 2008). Several studies demonstrate that serum CRP can be additively influenced by the IL-6–*CRP* gene–gene interaction (Paik *et al.*, 2007). Increased serum CRP in obese individuals was correlated with elevated secretion of IL-6 and TNF-α in adipocytes and predisposes to the chronic inflammatory state associated with T2DM (Fernandez-Real

and Pickup, 2008). Overall, evaluating the effect of inflammatory factors in T2DM may provide new public health approaches for disease prevention and a novel strategy for early detection. These markers are evidently related to risk of T2DM and can be employed in early detection of disease risk and as predictive measure of response to preventive intervention (Badawi *et al.*, 2010; Elgayar and Aboulsoud, 2013).

2.5 Obesity and diabetes

Obesity, a condition in which there is an excess of stored adipose tissue in the body (WHO/NUT/NCD/98, 1997), is the result of a growing exposure, or an over exposure, to environmental and behavioral risk factors, such as physical inactivity, poor diet and stress, all of which affect the phenotype of those who are genetically susceptible to obesity (Seidell *et al.*, 2001). Unfortunately, the current generation is entering adulthood with levels of obesity that have never been seen before (WHO., 2000b). Obesity is considered a global public health as well as an economic crisis (King and Rewers, 1993). Over 300 million people around the world are obese (WHO., 2000b) and according to the WHO, obesity has reached epidemic levels in the 21st century and is now the most common nutritional disorder in the western world (WHO/NUT/NCD/98, 1997). The coinciding of both the obesity epidemic and that of T2DM has led to the term "diabesity", to stress the importance of the relationship between the two diseases (Astrup and Finer, 2000). Obesity is strongly associated with risk factors for metabolic diseases (WHO/NUT/NCD/98, 1997) and is the best described risk factor for T2DM (Lundgren *et al.*, 1988). It is also associated with dyslipidemia, HPTN and some cancers (Pi-Sunyer, 2004). Obesity is associated with early characteristics of T2DM development, such as impaired glucose tolerance

(Kopelman, 2000), and is thought to be causally linked to T2DM through insulin resistance (Pi-Sunyer, 2004). Weight gain caused by excessive fat accumulation leads to a resistance to insulin through changes in endocrine activity (Federation, 2003), specifically through the increased release of non-esterified fatty acids and glycerol, hormones (e.g. leptin and adiponectin), and pro-inflammatory cytokines (TNF-α, IL-6) (Wellen and Hotamisligil, 2005) which in turn lead to an increased demand for the production of insulin in the pancreas (Federation, 2003). If this process is coupled with poorly functioning pancreatic beta cells, or the decreased insulin production which occurs with age, then blood glucose levels cannot be controlled (Kahn et al,2001) which eventually leads to the development of diabetes (Federation, 2003). In other words, diabetes arises when the pancreas is unable to sustain the body's requirement for insulin (Kahn *et al.*, 2006).

2.5.1 Indirect measurements of body composition

Anthropometry is defined as the measurement of body weight and body dimensions. It is used to reflect body fat and body fat distribution in large epidemiological studies or in clinical settings (Han *et al.*, 2006). It is a fast, easy, inexpensive and very widely used method of estimating body fat distribution (Pietrobelli *et al.*, 1998; Han *et al.*, 2006). Anthropometry is used to assess growth and development as well as to assess the risk of chronic disease associated with obesity and adipose tissue distribution (Seidell *et al.*, 2001). It is frequently used in epidemiologic and pathophysiological research involving health risk, health outcomes, body fat distribution, overweight and obesity (Seidell *et al.*, 2001), as it provides surrogate measures of adiposity and regional fat distribution (Pietrobelli *et al.*, 1998). A disadvantage of anthropometry is the technical skill required by the clinician to maintain both the accuracy and reproducibility of the measurements

and to minimize the errors of prediction (Pietrobelli *et al.*, 1998). However, even with highly trained personnel, any indirect measure of body composition or adipose tissue distribution will result in errors of prediction (Lukaski *et al.*, 1986). The validity of anthropometric measurements also tends to vary depending on a subject's age, sex, and ethnicity (Wang *et al.*, 2000). The two most widely used anthropometric measurements to classify obesity and central adiposity are body mass index (BMI) and waist circumference (WC), respectively (WHO., 2000b) although there remains controversy regarding which anthropometric measure provides the greatest predictive ability for disease risk (Piers *et al.*, 2000). Most studies found measures of central adiposity to be more significantly associated with T2DM, with waist circumference usually being a stronger predictor than waist-to-hip ratio. However, some studies found BMI to be the strongest predictor of T2DM with no added predictive benefit from adding further measures of central adiposity while others found predictive benefits from adding the waist circumference measure to BMI (Meisinger *et al.*, 2006). Waist-to-hip ratio (WHR) was found to be significantly associated with T2DM, but only when not compared to other anthropometric measures. Hip circumference (HC) and thigh circumference (TC) have been found to be associated with a lower risk of DM (Snijder *et al.*, 2003), while another study found no anthropometric measure to be clearly superior in T2DM prediction (Sargeant *et al.*, 2002). In studies that have compared measures according to ethnicity, results have been inconsistent. Waist-to-thigh ratio seems to play a role in T2DM in the Pima Indian population (Warne *et al.*, 1995; Tulloch-Reid *et al.*, 2003), but not in any other ethnic subgroup analysis, although BMI was equally as predictive in that population and there was no demonstrated benefit to adding other anthropometric measures to the model (Tulloch-Reid *et al.*, 2003). In one

Hispanic population study, anthropometry was found not to be significant after including glucose and insulin in the model (Haffner *et al.*, 1990), while others found central obesity to play a role in T2DM prediction (Wei *et al.*, 1997). In African American and non-Hispanic white population studies, there were no anthropometric measures found to be superior in predicting T2DM (Stevens *et al.*, 2001). When accounting for gender, there are mixed results on whether central adiposity or overall obesity measures are more predictive for women (Lundgren *et al.*, 1988), while for men WC is more predictive than WHR (Wang *et al.*, 2005; Group, 2006). Taken together, previous literature in the area of prediction of T2DM using anthropometric measures especially within ethnic subgroups highlight the inconsistency and the need for more research in the area.

2.5.1.1 BMI

The most common measure of overall obesity is Quetelet's Index, also known as body mass Index (BMI) which is measured according to the following formula: weight (kg) / height (m^2) (Pietrobelli *et al.*, 1998). A higher BMI is associated with increased fat stores, those both centrally and peripherally located, and also with increased musculature but not necessarily with increased VAT (Seidell *et al.*, 1988). BMI is used to classify protein energy malnutrition (Pietrobelli *et al.*, 1998) and to classify obesity internationally by the WHO according to the following categories (validated for Caucasian populations): Normal weight (18.9-24.9 kg/m2), overweight (25-29.9 kg/m2) and obese (>30 kg/m2) (WHO/NUT/NCD/98, 1997). BMI estimations can be further developed according to a subject's age, gender and ethnicity (Alvarez *et al.*, 2007). A potential limitation to BMI measurements is despite the fact that it accounts for height, it does not account for leg length and it has been shown that differences in

leg length or body build account for a different relationship between BMI and percent body fat (Deurenberg *et al.*, 1999). However, it is inexpensive and easily administered but has recently been shown to be less accurate in predicting disease than other measures of body fat estimation (Bray *et al.*, 2002) as it provides no information as to the distribution of adipose tissue (WHO/NUT/NCD/98, 1997).

2.5.1.2 Waist circumference (WC):

Measures of central obesity provide additional predictive information for the risk of disease, especially T2DM, beyond that which is provided by measures of overall obesity. This is especially the case among those in the upper extremes of central obesity distributions (Chan *et al.*, 1994). Waist circumference (WC) is an excellent indicator of abdominal adiposity (Ross *et al.*, 1992) and is an inexpensive, strongly correlated surrogate measure of the amount of visceral adipose tissue (Molarius and Seidell, 1998). It is also strongly related to abdominal subcutaneous fat, total abdominal fat as well as total body fat (Lean *et al.*, 1996). It is used in metabolic syndrome definitions and diabetes risk scores as a marker of central obesity (Parikh *et al.*, 2007). There are conflicting findings on whether WC is the single most accurate anthropometric measure of central obesity; however a majority of studies find WC to be more closely associated with central obesity as measured by CT scan than BMI, waist-hip ratio, sum of skinfolds, subscapular-triceps ratio, sagittal abdominal diameter, and %body fat (Pouliot *et al.*, 1994), and more predictive of metabolic disease risk and thus preferred over other anthropometric measures (Ho *et al.*, 2001; Mamtani and Kulkarni, 2005; Wang *et al.*, 2005), especially those measuring overall obesity (Carey *et al.*, 1997; Sargeant *et al.*, 2002; Jensen, 2006). It is hypothesized that despite the various suggested WC cut-off values, the girth measurement would be most effective if treated

as a continuous variable, with health risk increasing as the WC value increases, rather than as a categorical value as it is currently usually presented (Janssen *et al.*, 2004). However, using a cut off point may be more appropriate in a clinical setting. Also, there is some controversy as to the ideal location of the waist circumference measurement. Currently, there is no consensus for the optimal measurement protocol and no scientific rationale for any of the protocols recommended by principal health authorities, i.e. WHO (WHO., 2000a) or National Institutes of Health (NIH). There are three protocols which predominate the literature: measurement at the umbilicus, at the midpoint between the rib and iliac crest and the minimal waist circumference (Ross *et al.*, 2008). The WHO's guidelines measure the midpoint between the lower border of the rib cage and the iliac crest. The NIH guidelines are to measure the superior border of the iliac crest (Obesity *et al.*, 2000**).** Both use bony landmarks as a guide for measurement placement. A recent review of WC measurements by outcome (Tesfaye *et al.*, 2007) showed that in prospective associations of WC with cardiovascular disease (morbidity and mortality), measurement at the umbilicus was found to be significantly associated with the outcome more often than other measurement protocols. In prospective associations with T2DM, measurement at the midpoint was found to be significantly associated with T2DM most often, followed by umbilicus then minimum WC. However, the finding of the review was that WC measurement protocol was found to have no substantial influence on the association of WC with morbidity and mortality (Ross *et al.*, 2008).

2.5.1.3 Waist-hip ratio (WHR)

Waist-to-hip-ratio (WHR) is the ratio determined when a subject's WC is divided by their hip circumference (HC), and has been used frequently to identify those subjects

with a greater upper body versus lower body distribution of obesity and also as a surrogate method of estimating central adiposity (Vazquez *et al.*, 2007). WHR is associated with increased central fat distribution, increased visceral adipose tissue (VAT), decreased thigh muscle and reduced physical fitness (Seidell *et al.*, 1988). Some studies show a strong correlation of WHR to VAT (Ross *et al.*, 1992), while others show no association (Mamtani and Kulkarni, 2005), which could be explained by the attenuation of the apparent relationship between VAT and WHR after controlling for age and total adiposity (Ross *et al.*, 1992). WHR has been shown in most cases to be a weaker predictor of CAD, T2DM and dyslipidemia than BMI (Heymsfield *et al.*, 1990). Many studies have contrasted WHR and WC in T2DM prediction with WC usually being more significantly predictive of T2DM (Wei *et al.*, 1997; Karter *et al.*, 2005; Wang *et al.*, 2005; Group, 2006; Meisinger *et al.*, 2006), however some studies have found a greater prediction of T2DM from WHR than with WC (Rosenthal *et al.*, 2004).

2.6 The metabolic syndrome

The metabolic syndrome is a cluster of risk factors for cardiovascular disease (CVD), including abdominal obesity, elevated glucose, hypertension, elevated triglycerides and low levels of high density lipoprotein (HDL) cholesterol. The syndrome has received increased attention after practical and updated definitions by the Adult Treatment Panel III (ATP III) and the International Diabetes Federation (IDF) (Table 2.1) (Alexander *et al.*, 2003; Grundy *et al.*, 2005). Although other classifications exist, and the criteria vary to some degree, all definitions identify a population with increased risk for developing T2DM and CVD (Alberti and Zimmet, 1998; Einhorn *et al.*, 2003).

Table 2.1 Common definitions of the metabolic syndrome

WHO (1998)	**NCEP/ATP-III (2001/2005)**	**IDF (2005)**
Insulin resistance or diabetes/impaired glucose tolerance (IGT)/impaired fasting glucose (IFG)* plus 2 of:	At least 3 of the following:	Waist circumference _ 94 (men)/ 80 (women) cm*** plus 2 of:
Blood pressure _ 140/90 or Treatment	Waist circumference>102 (men)/88 cm (women)	Blood pressure > 130/85 mmHg or treatment.
Triglycerides _ 1.7 mmol/L or HDL < 0.9 (women)/1.0 (men) mmol/l.	Triglycerides > 1.7 mmol/L	Triglycerides > 1.7 mmol/L or treatment.
BMI _ 30 kg/m2 or waist/hip ratio > 0.9 (men)/0.85 (women)	HDL < 1.0 (men)/ 1.3 (women) mmol/L	HDL < 1.0 (men)/ 1,3 (women) mmol/L.
Microalbuminuria	Blood pressure > 130/85 mmHg or treatment.	Glucose > 5.6 mmol/L or treatment.
	Glucose > 5.6** mmol/L or treatment.	

Adapted from Grundy SM (2008).

Whereas the original definition by (Reaven, 1988), as well as the definition by the (WHO., 2000a) emphasised insulin resistance as mandatory for the diagnosis, no measure of insulin resistance is present in the updated definitions by (WHO., 2000a). Instead, the role of central obesity measured by waist circumference has been given

more attention, and is mandatory by the IDF criteria. A few comparative studies have aimed to compare the various definitions, and it seems that the IDF-definition identifies slightly more individuals with the syndrome (Nilsson *et al.*, 2007; Sandhofer *et al.*, 2007). Physical inactivity and increased caloric intake have led to an emerging epidemic of obesity. In the United States abdominal obesity has tripled during the past 40 years (Okosun *et al.*, 2004), more than 25% of the US population can be classified as having the metabolic syndrome (Li *et al.*, 2007), and the prevalence is increasing (Ford *et al.*, 2004). Depending on which classification that has been used, similar prevalence of the syndrome can be found in India and several countries in Europe, whereas the prevalence is even higher in some Latin-American countries (21 to 43%) and lower in South-East Asia (Grundy, 2008). The prevalence is also increasing with age, affecting >40% of US adults above the age of 60 years (Ford *et al.*, 2002). Similar age-related prevalence has been shown in a Norwegian cohort, increasing from 13% in the 20-29 year age group to 41% in the 70-79 years age group in men, and from 6% to 51% for women in the corresponding age groups (ATP III criteria) (Hildrum *et al.*, 2007). As all the individual components of the syndrome have been shown to increase the risk of CVD, including elevated fasting glucose (Levitan *et al.*, 2004), abdominal obesity (Rexrode *et al.*, 1998), hypertension (Lewington et al, 2002), elevated triglycerides (Sarwar *et al.*, 2007) and low levels of HDL cholesterol (Castelli *et al.*, 1986), it has been discussed if the metabolic syndrome is a useful clustering of risk factors (Kahn, 2007), and if the syndrome really exists (Grundy, 2006). Furthermore, different combinations of these components might identify very different phenotypes, although the diagnostic criteria of the syndrome are fulfilled (Després *et al.*, 2008).

TABLE 2.2 AMERICAN DIABETES ASSOCIATION CRITERIA FOR DIAGNOSIS OF DIABETES AND IMPAIRED GLUCOSE REGULATION.

Category	**Fasting plasma glucose**	**2-hour post-load plasma glucose**
Normal	< 5.6 mmol/L	< 7.8 mmol/L Impaired fasting
Impaired fasting glucose (IFG)	5.6-6.9 mmol/L	-
Impaired glucose tolerance (IGT)	-	7.8-11.0 mol/L
Diabetes mellitus	> 7.0 mmol/L	> 11.1 mmol/L

Adapted from Genuth S (2003)

As an example, elevated fasting glucose is a more useful marker for increased risk of diabetes mellitus than any of the other components (Wilson *et al.*, 2005). The metabolic syndrome is a strong predictor of T2DM, with an increased incidence rate of 5 to 7-fold (Grundy *et al.*, 2005; Wilson *et al.*, 2005). Indeed, the increased cardiovascular risk might develop as a continuum in parallel with increasing fasting glucose, from the normal range via impaired fasting glucose to overt diabetes mellitus (Table 2.2) (Haffner *et al.*, 1990). The risk of developing CVD is approximately doubled in the metabolic syndrome (Grundy, 2008). Importantly, subjects with the syndrome may be classified as having low risk of CVD by both the Framingham score and the European Systematic Coronary Risk Evaluation (SCORE) but still be at increased risk of subclinical atherosclerosis and cardiovascular events (Kullo *et al.*, 2004). In a recent meta-analysis including 43 cohorts, the relative risk for cardiovascular events and death was 1.78, with the highest risk in women (relative risk 2.63) (Gami *et al.*, 2007). After

adjustment for traditional risk factors like hypercholesterolemia and smoking, the syndrome was still associated with increased risk (relative risk 1.54).

2.6.1 The metabolic syndrome - a pro-inflammatory state

There is increasing evidence that the metabolic syndrome is associated with a chronic, low-grade inflammation (Grundy *et al.*, 2005). Several pro-inflammatory cytokines have been shown to be elevated in parallel with an increasing number of components of the syndrome, whereas the anti-inflammatory and adipocyte-specific substance adiponectin is consistently lower (Kowalska *et al.*, 2008). Furthermore, pro-inflammatory cytokines have been reported to induce insulin resistance in both adipose tissue and muscle (Hanley *et al.*, 2004). Moreover, increased levels of CRP, IL-6 and low levels of adiponectin have been shown to predict the development of T2DM (Pradhan *et al.*, 2001). Recently, also IL-18 was reported to predict T2DM (Thorand *et al.*, 2005). Some investigators have discussed that T2DM; metabolic syndrome and atherosclerosis are multifactorial conditions which appear to have a common inflammatory basis (Pradhan and Ridker, 2002). And as both inflammation and the metabolic syndrome are known risk factors for CVD, it is currently discussed if a measure of inflammation should be included in the definition of the syndrome (Grundy *et al.*, 2005; Haffner, 2006). So far, CRP has been the most likely candidate (Ridker *et al.*, 2004; Haffner, 2006). Cross-sectional and prospective studies have shown added prognostic information for cardiovascular risk stratification with CRP in populations with the metabolic syndrome (Malik *et al.*, 2005). However, a recent prospective study showed that although both CRP and the metabolic syndrome were independent predictors of CVD, the combination of the two did not increase the predictive value (Rutter *et al.*, 2004). The role of other pro-inflammatory markers in predicting CVD in

populations with the metabolic syndrome remains to be investigated. In addition to a pro-inflammatory state, the metabolic syndrome is frequently accompanied by a hypercoagulable state with increased plasma coagulation and reduced fibrinolysis (Palomo *et al.*, 2006). In particular, the main inhibitor of the fibrinolytic system, plasminogen activator inhibitor type-1 (PAI-1), has been consistently shown to be elevated in the metabolic syndrome (Alessi and Juhan-Vague, 2006). Thus, the metabolic syndrome can in part be considered both a pro-inflammatory and prothrombotic state (Grundy *et al.*, 2005).

2.6.2 Adipose tissue - an important source of inflammation

The classical perception of adipose tissue as a passive storage place of fatty acids has gradually been replaced by the notion of adipose tissue, and visceral fat in particular as an active endocrine organ. Visceral fat is now considered a central feature and potential cause of the metabolic syndrome (Després *et al.*, 2008), in part mediated by release of a large number of metabolically active substances known as adipokines. Adipokines are involved in several biological processes, including inflammation, thrombosis, insulin sensitivity and energy balance (Lau *et al.*, 2005). Not only adipocytes, but also stromal cells such as macrophages, fibroblasts and endothelial cells are involved in the production of various adipokines (Hajer *et al.*, 2008). Several proinflammatory markers have been shown to be produced in adipose tissue, including MCP-1, TNF-_, IL-1, IL-6, IL-8 and IL-18 (Fain *et al.*, 2006; Gustafson *et al.*, 2007). In the presence of obesity, adipose tissue produces cytokines in excess, whereas the production of adiponectin is diminished, thus shifting the balance to a pro-inflammatory state (You *et al.*, 2005). Whether systemic inflammation depends mostly on the quantity or the quality of adipose tissue is not known, but probably the quantity of visceral adipose tissue is

important (Hajer *et al.,* 2008). In contrast to subcutaneous fat, visceral fat drains directly to the portal circulation, and a study in extremely obese patients indicated that visceral fat was the main contributor of plasma IL-6 levels (Fontana *et al.,* 2007). Thus, in obese people, it is likely that viscerally produced IL-6 drained via the portal circulation could be an important inductor of CRP-production in the liver (Hajer *et al.,* 2008). Moreover, IL-6 has been shown to induce Plasminogen activator inhibitor type 1 (PAI-1) production in visceral and subcutaneous adipose tissue (Alessi *et al.,* 1997). PAI-1 is produced in substantial amounts in human adipose tissue (Alessi *et al.,* 1997), and in a study of obese patients abdominal visceral fat expressed 5-fold more PAI-1 than subcutaneous fat (Bastelica *et al.,* 2002). Hence, adipose tissue is likely to be a major contributor to the pro-inflammatory, pro-thrombotic state characteristic of the metabolic syndrome.

2.7 Lipid components in diabetic patients

2.7.1 Serum Total Lipids:

Lipids are important dietary constituents, not only for their high energy value, but also because of the fat-soluble vitamins and essential fatty acids contained in the fat of natural foods. Lipid metabolic abnormalities play an important role in various diseases such as hyperlipidemia, heart disease and diabetes mellitus (Liebich, 1986)**.** Diabetes and hyperlipidemia are frequently associated pathologic states. Diabetes is considered causative or aggravating factors in hyperlipidemia. Yet, the incidence of hyperlipidemia is high in this state, and the severity of disturbed metabolism differs from one patient to another (Debry, 1979)**.** Hyperlipidemia is the result of an imbalance between the formation and degradation of either the lipoprotein entity or any of its constituents. The

level of serum lipids are affected by a multitude of factors such as race, heredity, age, sex, hormones, diet, physical activity, season and method of analysis (Larsson *et al.*, 1962). Few of the materials in the literature are uniform in these respects and any detailed comparison between the results of different investigators would therefore be of limited value. Of interest to mention, is that serum lipids are different among population of different countries. Thus in diabetics Senegalese, serum lipids and its components were increased in relation to that of healthy Senegalese and have the same levels as healthy Europeans (Josselin *et al.*, 1975). The most common lipid abnormality in diabetic is hypertriglycerdemia (Bagdade *et al.*, 1968), but plasma cholesterol also can be increased. In addition, the chemical composition of lipoproteins is abnormal (Schonfeld *et al.*, 1974)**.**

2.7.2 Serum triglycerides:

Triglycerides are stored in adipose and act as a large energy reserve, which can be made available when required by enzymatic hydrolysis to fatty acids and glycerol. About 30-40% of people's daily caloric intake is normally in the form of fat. After hydrolysis, the dietary fat is absorbed primarily as monoglycerides and fatty acids, and resynthesized into triglycerides in the mucosal cells. The triglycerides are then combined with cholesterol, phospholipids and apolipoprotein and secreted into the lymph system as chylomicrons. The amount of fat in a meal appears to determine the amount of triglycerides resyntheslized in the mucosal cells, the more the latter, the higher and the proportional of chylomicrons to VLDL (Gangl and Ockner, 1975). It has been established that diabetes is often associated with increased plasma triglyceride level (Laakso *et al.*, 1985; laakso *et al.*, 1987). In insulin- dependent diabetes mellitus (Juvenile-onset diabetes), the change in both males and females was insignificant

(Beach *et al.,* 1979). Serum triglyceride level was significantly higher in alloxan-diabetic rabbits than in non-diabetic rabbits and in young alloxan diabetic rats than in non-diabetic rats (Saatov, 1980). Multiple mechanisms may be responsible for the increase of serum triglyceride level in diabetes. In absolute insulin-deficiency, there is an increased concentration of serum free acids with increased endogenous synthesis of triglycerides (Nikkilä and Kekki, 1973) and a decreased activity of adipose tissue lipoprotein lipase and post heparin lipoprotein lipase (Bagdade *et al.*, 1968), which provide an adequate explanation for hypertriglyceridemia.

2.7.3 Serum total cholesterol:

The normal human body contains about 2 gm of cholesterol per Kg. total body weight, but only about 5% of this value is present in the plasma lipoproteins. Almost all animal tissues are capable of synthesizing cholesterol from acetate, but the most activity synthesizing sites are the liver and gastrointestinal tract (Grundy, 1978). However, it was reported that, the mean level of serum cholesterol was significantly higher in normal girls than in normal boys in Washington and Sanghai (Zhijia, 1986). These authors believed that cholesterol metabolism is influenced by hormones during the adolescent period. Serum cholesterol in Egyptian male normal children (6-12 years) was insignificantly higher than in corresponding females (Sabry, 1983b)**.** However, (Wilding *et al.*, 1972) reported that in healthy subjects, serum cholesterol concentration is significantly higher in male than in female. The mean value for serum cholesterol was found to be significantly increased with age (Zhuang *et al.*, 1986)**.** An increase in blood cholesterol normally occurs after end of the adolescent period (Wilding *et al.*, 1972). Several studies confirmed that plasma cholesterol is elevated in diabetic populations and there is evidence that other aspects of cholesterol metabolism are abnormal (Florey

et al., 1973). This may play a role in the accelerated development of the arteriosclerotic vascular disease that is a major long-term complication of diabetes in humans (Palumbo *et al.*, 1976). The concentration of serum cholesterol was increased in diabetic patients (Lowy *et al.*, 1958*).* However, other authors compared diabetic and non diabetic participants and found no difference in the concentration of serum cholesterol (Briones *et al.*, 1984).

2.8 HYPERTENSION

2.8.1 Definition and Diagnosis

Hypertension is a medical condition in which the blood pressure is chronically elevated (Mandal, 2009).

WHO criteria for hypertension:-

The systolic blood pressure (SBP) of a person is consistently 140mmHg or greater and the diastolic blood pressure (DBP) is consistently 90mmHg or greater is considered as a case of HPTN. It is diagnosed by measuring the blood pressure by mercury sphygmomanometer, either by the palpatory or auscultatory method. Blood pressure can be measured in supine, sitting or standing position but the arm should be at the level of heart. Before the measurement of blood pressure is taken, the patient should be kept in rest at least 5 minutes in quiet room in comfortable sitting position with arm muscles relaxed. A cuff applied should be of suitable size and is to be evenly applied. Both SBP and DBP should be measured at least twice over a period of not less than three minutes. Both readings should be recommended and the mean value for each is calculated which is considered as result (Green and Kreuter, 1999)**.**

2.8.2 CLASSIFICATION OF HYPERTENSION

Taking into consideration associated risk factors and development of HPTN-related organ damage, the classification of HPTN provides an easy and reliable method of assessing risk and the most appropriate treatment for each patient. HPTN can therefore be classified in three ways by: etiology, blood pressure level and extent of damage of the organs (Aalto, 1999).

2.8.2.1 On Etiological Basis

There are three types of HPTN (Green and Kreuter, 1999)

1. Essential (primary) HPTN: where the specific cause is not known.

2. Secondary HPTN: where hypertension is the result of other conditions like disease of kidney or tumours (eg pheochromocytoma and paraganglioma).

3. Resistant HPTN: where a person's blood pressure remains above their target blood pressure despite taking three or more medications to lower it, it is considered as resistant hypertension.

2.8.2.2 By Blood Pressure level

As per WHO/ISH and ESH-ESC guidelines (WHO/NUT/NCD/98, 1997; Narkiewicz, 2006).

Table 2.3 Classification of hypertension by blood pressure levels

Category	SBP(mmHg)	DBP(mmHg)
Optimal	<120	<80
Normal	120-129	80-84
High normal	130-139	85-89
Hypertension		
Grade I(mild)	140-159	90-99
Grade II(moderate)	160-179	100-109
Grade III(severe)	≥180	≥110
Isolated systolic	≥140	<90
Sub group-borderline	140-160	<90

Adapted from Ganesh Kumar Mandal (2009)

2.8.3 Independent Variables

2.8.3.1 Age

Blood pressure tends to rise in elderly people. But increased blood pressure is not a routine part of ageing. Ageing is not only a risk factor of HPTN but also the risk factor of many other diseases. In fact, it is not a cause of HPTN but elderly people are more vulnerable to this disease. Isolated HPTN is the commonest type of high blood pressure in older adults (Health and Services). Many studies have shown that there is positive relationship between age and HPTN. Cross-sectional surveys as well as prospective observational cohort studies have consistently demonstrated that there is a positive relation between age and blood pressure with diverse geographical, cultural and socioeconomic characteristics (Whelton *et al.*, 2004). Most populations in industrialized countries show an increase in SBP and DBP with age, but with DBP decreasing or reaching a plateau. DBP also tends to rise with age but at slower rate than SBP. Increased blood pressure with age is attributed to the effects of environmental factors

such as sensitivity to salt, increased BMI, and alcohol consumption (Kornitzer *et al.*, 1999).

2.8.3.2 Body Mass Index (BMI)

BMI is an index of weight-for-height that is commonly used to classify underweight, overweight and obesity. Chronic imbalance between energy intake and actual requirement of energy by the body can cause overweight and obesity (WHO., 2003). Overweight and obese people are more likely to develop HPTN (Health and Services). The prevalence of HPTN is significantly higher in people with obesity and android obesity. The BMI is used to define obesity (BMI>30kg/m^2), overweight (BMI> 25kg/m^2) and the waist to hip ratio (WHR) is used to define android obesity (WHR>0.9) (Ghannem and Fredj, 1999). Some of the causes of HPTN are increased physical inactivity and consumption of foods rich in saturated fatty acid and sugar (WHO., 2003). There is a shift in the burden of obesity and overweight towards lower socioeconomic groups because of their lifestyle changes. Studies have shown that proportion of overweight people has exceeded the proportion of underweight people in majority of countries. The median ratio of overweight to underweight was 5.8 in urban areas and 2.1 in rural areas. According to the recent burden of disease estimates from WHO, overweight and HPTN are among the top three leading causes of disease burden among women and among the top five leading causes of disease burden among men in central Asia (Mendis *et al.*, 2007). About one in three adults are overweight or obese, and cardiovascular disease accounts for about two-thirds of all deaths. Results of several studies show that obesity is associated with eating animal source protein and having smoked in the past. Obese men and women are at three times greater risk than those with normal BMI, without any dependence on physical activity, dietary habit,

smoking, use of alcohol and other factors. In women, the possible relationship between hypertension and obesity was observed at all levels of BMI whereas for men it was only associated with BMI above 25 Kg/m^2 (Mendis *et al.*, 2007). Management of HPTN in obese individuals is more complicated because of poor response to treatment and also increased need for multiple medications. Epidemiological studies have shown that the age adjusted prevalence of HPTN increases with higher levels of BMI. The linkage between blood pressure and BMI is stronger for SBP than DBP. Central obesity has a stronger effect on high blood pressure than lower body obesity. Blood pressure appears highest in those with a high waist and small hip circumference (Narkiewicz, 2006). Obesity and HPTN can lead to the development of left ventricular hypertrophy (LVH). They have an additive effect in men and synergistic effect in women. Hence obese hypertensive women are at higher risk of developing LVH. Several mechanisms have been proposed which show the relationship between obesity and HPTN. These are alteration in the rennin-angiotensin-aldosterone system, increased activity of the sympathetic nervous system, insulin resistance, leptin resistance, altered coagulation factors, dysfunction of endothelium, and inflammation of endothelium. Obesity can lead to the development of HPTN by increasing renal sodium absorption, impairing pressure natriuresis, and volume expansion. It may cause chronic renal failure by changing the renal structure. Insulin resistance syndrome (IRS) may cause volume expansion, sodium retention and enhancement of sympathetic nervous system activity. IRS in association with obesity may increase rennin-angiotensin system activity that leads to increased cardiovascular risk. Obesity is also associated with haemodynamic alteration. Although both cardiac output and plasma volume increase, there is significant reduction in peripheral resistance in obese people compared with normal

weight individuals (Narkiewicz, 2006). In many epidemiological studies, a clinically significantly correlation between BMI and hypertension has been observed (Kornitzer *et al.,* 1999). Some evidence from cross sectional and prospective observational studies has shown that there is a direct, strong and consistent relationship between body weight and blood pressure. Overweight is associated with 2 to 6 fold increase in the risk of developing hypertension (Macmohan *et al.,* 1987)**.** Higher BMI is associated with higher SBP and DBP (Ribeiro *et al.,* 2003).

TABLE 2.4 CLASSIFICATION OF WEIGHT BY BMI IN ADULTS ACCORDING TO WHO

Classification	**BMI(Kg/m^2)**
Underweight	<18.5
Normal weight	18.5-24.9
Overweight	≥25.0
Pre-obese	25.0-29.9
Obese	≥30.0
Obese class I	30.0-34.9
Obese class II	≥35.0-39.9
Obese class III	≥40.0

Adapted from Ganesh Kumar Mandal (2009)

2.8.3.3 Physical Activity

Physical activity is essential in improving physical and mental health of people. It reduces the risk of non-communicable diseases like HPTN, heart disease, diabetes mellitus, and also increases social interaction by community engagement. It is not only related to the public health issue but also to the promotion of general well being. Study suggests that in European region, physical inactivity is responsible for 600,000 deaths (about 6% of total deaths). Physical activity differs among different socio-economic

groups. It suggests that people with low socioeconomic status have less leisure time and live in environment that do not support physical activity. There is an inverse relationship between physical activity and prevalence or incidence of HPTN. Low leisure time physical inactivity increases the risk of HPTN. Physical fitness is inversely related to blood pressure and incidence of HPTN (Kornitzer *et al.*, 1999). In post menopausal women with moderate HPTN, regular aerobic exercise significantly lowered blood pressure after 12 weeks (Kornitzer *et al.*, 1999). Sedentary and unfit normotensive individuals have a 20-50% higher risk of developing HPTN than active and fit people. Regular aerobic physical activity has been shown to be beneficial for both prevention and treatment of HPTN (WHO/NUT/NCD/98, 1997). At least 30 minutes per day of regular moderate intensive physical activity is recommended. This will lead to burning of 150 k cal of energy in adults. Exercise lowers SBP and DBP by 5-10mmHg (WHO/NUT/NCD/98, 1997). Dynamic exercise is more effective compared with static exercise. It is necessary to do very hard and strenuous exercise. Previous research has shown that a mean reduction in 6.4mmHg of SBP and 6.9mmHg of DBP is achieved by regular exercise (WHO/NUT/NCD/98, 1997).

2.9 Inflammation and Oxidative Stress in Hypertension

Chronic inflammation can also trigger oxidative stress, which has been associated with HPTN (Crowley, 2014). As mentioned, inflammation is the primary immune response to eliminate pathogens or to repair tissue damage. Innate immune cells, such as neutrophils and macrophages, produce reactive oxygen species (ROS) such as superoxide and hydrogen peroxide in order to kill pathogens (Crowley, 2014). Nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) is a major source of

ROS in immune cells and also in the vasculature (Drummond *et al.*, 2011). Inflammatory processes continue until the pathogens are destroyed or the tissue repair process has been completed. However, sustained inflammation can lead to an overproduction of ROS. Oxidative stress (defined as an imbalance between the production and breakdown of ROS) is a major cause of endothelial dysfunction, primarily through reducing NO bioavailability via the direct chemical reaction of superoxide with NO, resulting in the formation of peroxynitrite (Touyz, 2004). The reaction between superoxide and NO is faster (Huie and Padmaja, 1993) than the breakdown of superoxide via superoxide dismutase (Huie and Padmaja, 1993). Furthermore, peroxynitrite formation may result in further impairment of NO levels and enhanced oxidative stress by inhibiting eNOS activity through oxidation of 4-tetrahydrobiopterin (BH4), a cofactor of eNOS. This leads to eNOS uncoupling, where eNOS produces superoxide instead of NO (Vásquez-Vivar *et al.*, 1998). Excessive ROS levels can also induce cellular damage by interacting with DNA, lipids, and proteins (Hassoun *et al.*, 2001), which may further impair vascular structure and function. Immune cells such as T cells, macrophages, and neutrophils express NADPH oxidase subunits and produce ROS. In the setting of Ang II-induced hypertension, T cells express higher levels of p47phox, p22phox, and NOX2, components of NOX2 oxidase. Furthermore, adoptive transfer of T cells deficient in NADPH oxidase results in lower superoxide production and HPTN in response to Ang II (Guzik *et al.*, 2007). Oxidative stress can promote inflammatory processes by activating transcription factors such as NF-κB (Canty *et al.*, 1999). CRP levels have been shown to correlate with the level of oxidative stress in inflammatory cells from hypertensive patients (Yasunari *et al.*, 2002).

Inflammatory Markers, Clinical Outcome and Therapeutic Benefits in Cardiovascular Patients

2.9.1 hs- CRP:

Endothelial damage/dysfunction is associated with markers of inflammation in patients with HPTN and/or diabetes. C-reactive protein(CRP) has been related to insulin resistance, systolic BP, pulse pressure and HPTN (Chae *et al.*, 2001; Engström *et al.*, 2002), and to markers of endothelial dysfunction (plasma levels of vWF, tissue plasminogen activator and cellular fibronectin) (Yudkin *et al.*, 1999). Elevated levels of CRP predict development of the metabolic syndrome, at least in women (Han *et al.*, 2002). This association is even stronger when combined with BMI (body mass index). CRP and PAI-1 levels are elevated in subjects with insulin resistance, with levels that are higher than in patients with coronary artery disease (Festa *et al.*, 2003). Thus low-grade inflammation in the pre-diabetic state is associated with increased insulin resistance. Although subjects with insulin resistance had greater adiposity, levels of CRP were not influenced by BMI. However, hs-CRP levels were higher among the high-BMI subgroup of subjects who did not develop diabetes during follow-up period. Numerous epidemiological studies have shown that serum hs-CRP level is a powerful predictor of ischaemic cardiovascular events in patients with stable or unstable angina, appears to correlate with softer plaques that are more prone to rupture, and may even predict cardiovascular events among apparently healthy subjects (Ridker *et al.*, 1998). It may thus be useful in targeting medium-risk patients who could benefit from aggressive cardiovascular preventative therapy. Furthermore, CRP levels are positively associated with systolic BP, pulse pressure and incident HPTN (Chae *et al.*, 2001). Thus CRP and

high BP in combination have additional predictive value for cardiovascular outcomes, as they contribute as independent determinants of cardiovascular risk. Hs-CRP may be more than an inflammatory marker of increased cardiovascular risk. CRP has been demonstrated in atherosclerotic plaques and appears to be involved in foam cell formation, promotes monocyte chemotaxis and facilitates LDL (low-density lipoprotein) uptake by macrophages in vitro (Torzewski *et al.*, 2000; Zwaka *et al.*, 2001). In endothelial cells, CRP facilitated the release of PAI-1 [64] and ET-1 (Verma *et al.*, 2002), and increased the expression of cell adhesion molecules (Pasceri *et al.*, 2000), reduced NO bioavailability (Venugopal *et al.*, 2002) and NO-mediated dilation in the vasculature. In VSMCs, CRP induced the expression of AT1 receptors and enhanced AT1 receptor-mediated ROS formation, which reduced NO bioavailability (Verma *et al.*, 2002) and activated stress-activated p38MAPK and JNK (Wang *et al.*, 2003). However, CRP may also have anti-inflammatory actions by inhibiting neutrophil activation and adhesion (Zouki *et al.*, 1997) and blocking platelet aggregation in vitro (Fiedel and Gewurz, 1976). A Mediterranean-style diet has beneficial effects on endothelial function and vascular inflammatory markers in patients with metabolic syndrome. Patients consuming a Mediterranean-style diet had a significant reduction in serum concentration of hs-CRP, IL-6, IL-7 and IL-18, as well as in insulin resistance (Esposito *et al.*, 2004). Exercise training, together with weight loss, reduce CRP levels significantly, although not in proportion to weight reduction (Obisesan *et al.*, 2004). CRP decrease was observed in the middle weight reduction quartile, suggesting that there may be an optimal link between exercise and weight loss with respect to the inflammatory status (Obisesan *et al.*, 2004). In patients with high

LDL-cholesterol levels, those with low CRP have better clinical outcomes than those with higher levels (Ridker *et al.*, 2004; Nissen *et al.*, 2005).

Adapted from C. Savoia and E. L. Schiffrin (2007)

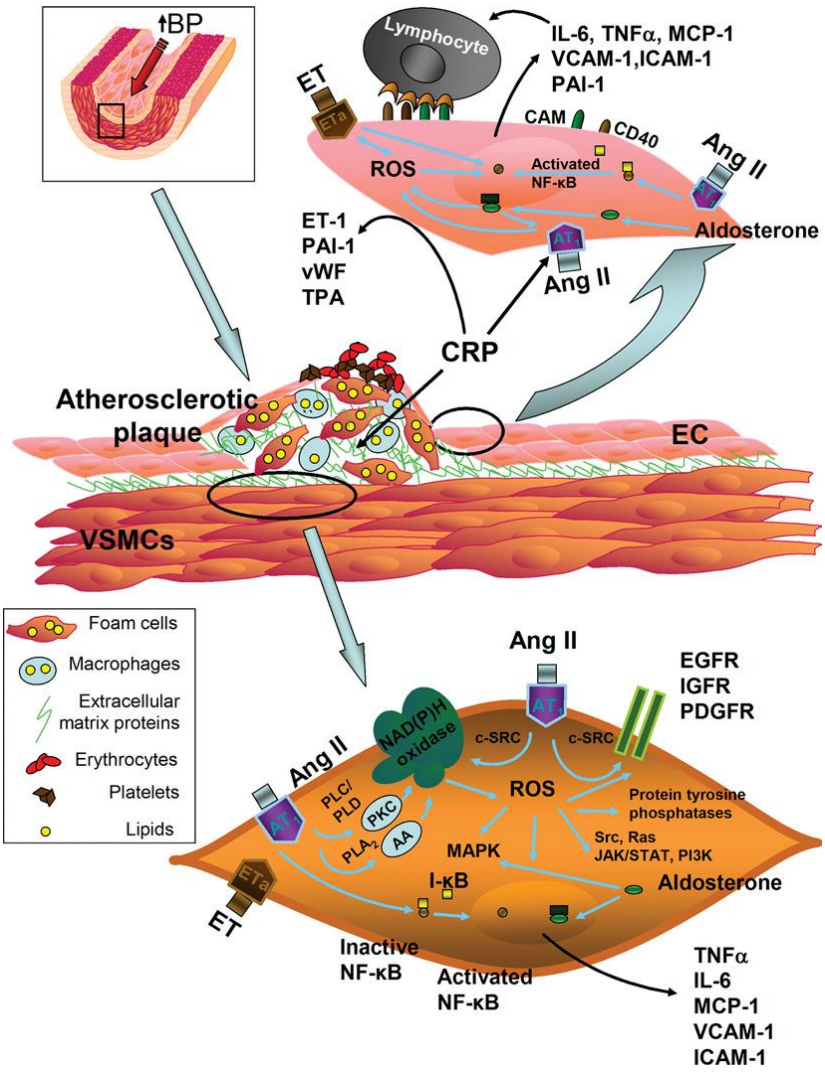


Figure 2.1 Molecular mechanisms of vascular inflammation

Abbreviations: AA, arachidonic acid; CAM: cell adhesion molecule (ICAM-1 and VCAM-1); EC, endothelial cells; EGFR, epidermal growth factor receptor; IGFR, insulin-like growth factor receptor; I-κB, inhibitory κB; JAK, Janus kinases; PDGFR, platelet-derived growth factor receptor; PI3K, phosphoinositide 3-kinase; PKC, protein kinase C; PLA2, phospholipase # A2; PLC, phospholipase C; PLD, phospholipase D; STAT, signal transducers and activators of transcription; TPA, Tissue plasminogen activator

2.9.2 Cytokines (TNF-α and ILs)

TNF-α is a primary inflammatory cytokine secreted by several cell types involved in vascular inflammation (endothelial cells, VSMCs and macrophages). It enhances monocyte recruitment into atherosclerotic lesions in early stage atherosclerosis (Niemann-Jönsson *et al.*, 2000). Patients with increased risk of recurrent coronary events have persistently elevated plasma levels of TNF-α. Weight loss in obese patients reduce plasma levels of TNF-α (Monzillo *et al.*, 2003). In patients with hyperlipidaemia, treatment with simvastatin and the PPAR-α agonist fenofibrate significantly reduce plasma levels of TNF-α (Koh *et al.*, 2002). Moreover, in patients with HPTN or obese subjects, candesartan or rosiglitazone lowered TNF-α (Koh *et al.*, 2004). IL-1 is an inflammatory cytokine involved in the early stages of the inflammatory process. Plasma levels of IL-1 are elevated in patients with coronary artery disease (Koh *et al.*, 2004**).** IL-6 is a secondary inflammatory cytokine which induces the increase of plasma concentrations of fibrinogen, PAI-1 and CRP and, in healthy men, elevated levels of IL-6 are associated with increased risk of future myocardial infarction (Ridker *et al.*, 2000). IL-18 is an IFN-γ (interferon-γ)- inducing factor, which is one of the strongest predictors of cardiovascular death as well as of development of Type 2 diabetes (Fischer *et al.*, 2005; Thorand *et al.*, 2005). Weight loss, a Mediterranean style diet and exercise reduce serum concentrations of IL-6, IL-7 and IL-18 in obese subjects (Esposito *et al.*, 2004). Statins induce a significant reduction in IL-1 and IL-6 levels (Koh *et al.*, 2002). Inhibition of RAAS with either enalapril or losartan in patients with stable angina contributed to a decrease in the release of IL-1 and IL-6 (Trevelyan *et al.*, 2004). PPAR-α ligands inhibit production of IL-6 as well as prostaglandins and COX-2. These effects occur as a consequence of

PPAR-α-induced inhibition of signaling by the pro-inflammatory mediator NF-κB and induction of apoptosis (Staels *et al.*, 1998). The action of PPAR-γ activators on inflammatory markers is a matter of controversy. Some studies have demonstrated that PPAR-γ activators inhibit the expression of TNF-α, IL-1 and IL-6 (Jiang *et al.*, 1998). On the other hand, rosiglitazone did not reduce IL-6 levels in patients with Type 2 diabetes, and may enhance inflammatory responses in epithelial cells by potentiating TNF-α-induced production of IL-6 and/or IL-8 (Desmet *et al.*, 2005), although it reduces CRP levels (Jiang *et al.*, 1998; Haffner *et al.*, 2002). Nevertheless, several studies have demonstrated that activation of PPAR-γ exerts anti-atherosclerotic actions (Ishibashi *et al.*, 2002).

2.9.4 Overweight, Obesity and Hypertension

Chiang and co-workers (Chiang *et al.*, 1969) define overweight as the ratio of actual weight to average or desirable weight (specific for age, sex, height, and body build). They add that an individual could be overweight on account of musculature or bony structure rather than excess fat, so that overweight and obesity are not necessarily synonymous. Weight gain in adults has a detrimental impact on physical health and can lead to death (MacKnight, 2003). Its side effects in human body can range from psychological effects that impact quality of life (e.g. poor self-esteem, discrimination, depression) to physiological conditions that subject an individual at risk for premature deaths (Guilbert, 2003; Keller, 2008). A person may develop one or combinations of two or more of the following conditions: HPTN, dyslipidemia (high total cholesterol and high levels of triglycerides),T2DM, heart disease, stroke, gallbladder disease, osteoarthritis, sleep apnea and respiratory problems, some cancers (such as endometrial, breast, and colon)(Guilbert, 2003). Most health practioners believe that the more

overweight an individual is, the higher the risk for developing health complications, the converse also appears true. Body weight plays a significant role in BP. The relationship between body weight and BP has been documented in several other studies (Zein and Assefa, 1986; Poulter *et al.*, 1990). Weight reduction of as little as 4.5kg can produce a decrease in BP in a large proportion of overweight people with HPTN (Porth and Kunert, 2002). In another study it was reported that for each 10kg increase in weight in western population there was an increase of 2-3 mmHg in SBP and 1-3mmHG in DBP (Deshmukh *et al.*, 2006). The anatomic distribution of weight is a factor in determining which people are more susceptible to HPTN (White *et al.*, 1986). Literature reveals through both cross-sectional and longitudinal studies that there is a significant association between relative weight and HPTN. Chiang and the colleagues (Chiang *et al.*, 1969) assert that there is a frequent coexistence between overweight and HPTN which suggests a causal relationship between the two conditions. They further add that weight gain constitutes one kind of environmental stress that brings predisposition toward HPTN into the open. HPTN is said to be approximately twice more prevalent in the obese than in the non-obese (Kotchen, 2008). Increasing evidence accumulated through both metabolic and epidemiologic studies indicate that where excessive fat is located on the body, one's health is at risk (Pi-Sunyer, 2004). Body fat distribution has been suggested to be the most indicator of HPTN risk than actual overweight (Porth and Kunert, 2002). It is asserted that aberrant fat localisation, such as abdominal adiposity, and not total fat mass, is the most crucial determinant of cardiovascular diseases (Forsyth, 1969). In most epidemiological studies, anthropometric measurements have been used to assess the fat distribution and the relationship between the body fat distribution indices and the risk of hypertension (Guagnano *et al.*, 2001).

Anthropometric indicators that reflect excess adipose tissue are ;BMI, which reflects the proportion of adipose tissue in the total body mass, regardless of localisation-and the WHR-which provides a measure especially of visceral fat (Sarno and Monteiro, 2007).

2.9.5 Anthropometric measurement of overweight, obesity and Blood Pressure

2.9.5.1 Body Mass Index (BMI)

Body mass index is an anthropometric index for measuring overweight and obesity among adults (Keller, 2008). Keller emphasizes that BMI should be used as a suggestive indicator of risk and not an absolute tool for medical diagnosis. She further adds that its categories have more correlations with disease risk, the higher BMI the higher the risk for CVD and all cause of mortality in both men and women (Sharma and Chetty, 2005).There is a relationship between BMI values, morbidity and mortality.BMI values of 20-25 kgm^{-2} represent a range associated with good health for most people, while BMI values below $20kgm^{-2}$ and greater than $27kg^{-2}$ can be associated with health problems (Sharma and Chetty, 2005). Positive associations between BMI and BP have been documented in several studies (Edwards *et al.*, 2000). While some studies (Cassano *et al.*, 1990) reported a consistent, and modest association between BMI and BP, others suggest a BMI threshold at which level the relationship with BP should begin (Van Dyke and Dave, 2005). In Tanzania, NJelekela and co-workers reported a significant correlation of BMI to SBP and DBP, in both genders, (Njelekela *et al.*, 2001; Njelekela *et al.*, 2003), this was also observed in Nigeria by Kadiri and co-workers (Kadiri *et al.*, 1999). However, white and co-workers reported that, for Canadian men, HPTN was more highly correlated with BMI in almost every age group while (White *et al.*, 1986; Tesfaye *et al.*, 2007) concluded from their study that the relationship between BP and BMI is U or J shaped, an observation which was

suggested before, although inconsistent (Baldinger *et al.*, 2005). From their study which involved subjects from Vietnam, Indonesia and Ethiopia, Tesfaye and co-workers also noticed prevalence of high blood pressure in both underweight and overweight population subjects and the relatively low blood pressure in the normal range BMI range. Despite several early studies report, and a strong relationship between BMI and BP, the perception today is different due to new reports of increased BP and HPTN prevalence rates in lean populations (Tesfaye *et al.*, 2007). Several studies have shown that the relationship between body fat and BMI cutoffs points differ between populations, and attribute the differences to body composition based on genetics, ethnic background as well as energy intake and physical activity (Beevers *et al.*, 2001). Following this difference several countries have developed BMI-for-age charts for their populations and some have gone further into defining cutoffs points on these charts to define overweight and obesity in different populations (WHO., 2000b). Africa has not developed any and still relies on the BMI cutoff points proposed by the WHO (WHO/NUT/NCD/98, 1997) as the universal standard for classification of obesity (Table 2.5). The use of BMI as an assessment of adiposity is non intrusive, reliable and has been validated against measures of body density (Bellizzi and Dietz, 1999), however. several limitations; including its inability to distinguish between fat mass and non-fat mass as well as abdominal visceral and subcutaneous fat have been reported (WHO., 2000b).

TABLE 2.5 THE INTERNATIONAL CLASSIFICATION OF ADULT UNDERWEIGHT, OVERWEIGHT AND OBESITY ACCORDING TO BMI

Classification	BMI(kg/m^2)	
	Principal Cut-offs points	Additional cut-off points
Underweight	<18.50	<18.50
Severe thinness	<16.00	<16.00
Moderate thinness	16.00-16.99	16.00-16.99
Mild thinness	17.00-18.49	17.00-18.49
Normal range	18.50-24.99	18.50-22.99
		23.00-24.99
Overweight	≥25.00	≥25.00
Pre-obese	25.00-29.99	25.00-27.49
		27.50-29.99
Obese	≥30.00	≥30.00
Obese class I	30.00-34.99	30.00-32.49
		32.50-34.99
Obese class II	35.00-39.99	35.00-37.49
		37.50-39.99
Obese class III	≥40.00	≥40.00

Adapted from the WHO Global Database on Body Mass Index (BMI)

2.9.5.2 Waist to Hip Ratio (WHR):

Central obesity is often referred to as abdominal, upper-body, male-type. android, or visceral obesity vs. female-type or gynoid obesity, where there is preferential fat accumulation in the gluteal and femoral distribution (Aneja *et al.*, 2004). To differentiate between central or upper body obesity (i.e. fat cell deposits in the abdomen) from lower body obesity with fat cell deposits in the buttocks and legs, WHR is commonly used (White *et al.*, 1986). WHR has been long recognised as a substantial component in the assessment of cardiovascular disease risk factors due to a positive

association between high WHR and HPTN (Porth and Kunert, 2002; Shahbazpour, 2003). Its validity as a measure of abdominal obesity has been evaluated in several studies by comparing WHR with abdominal fat measured by computed tomography (Willett, 1998). The index is capable of predicting the risk of obesity related morbidity and mortality as they account for regional abdominal adiposity. Evidence exist suggesting that obesity and High BP are disorders that are closely linked, particularly when obesity is characterised by a central fat distribution, (Kelley, 1999), a type of fat reported to be more insulin resistant than peripheral fat (Porth and Kunert, 2002). The abdominal accumulation of body fat, apart from overall level of adiposity, is associated with increased BP, an increased risk of HPTN, (Cassano *et al.*, 1990) and many other metabolic abnormalities that are generally regarded as part of insulin resistance syndrome (IRS) (SWAI *et al.*, 1993). WHO in 1998 provided a chart which shows general guidelines for acceptable levels for waist-hip ratio (see table below). Individuals with either higher BMI or central adiposity distribution are potential candidates at increased risk of HPTN and cardiovascular disease (Shahbazpour, 2003).

TABLE 2.6 GENERAL GUIDELINE FOR ACCEPTABLE LEVELS FOR WAIST-HIP RATIO

	Acceptable		**Unacceptable**		
	Excellent	**Good**	**Average**	**High**	**Extreme**
Male	<0.85	0.85-0.90	0.90-0.95	0.95-1.00	>1.00
female	<0.75	0.75-0.80	0.80-0.85	0.85-0.90	>0.90

Adapted from the WHO Global Database on Body Mass Index (BMI)

2.9.5.3 Biochemical markers

2.9.5.3.1 Glucose:

T2DM is a disease associated with abnormal carbohydrate metabolism which arises due to insulin deficiency as insulin is the key hormone responsible for glucose homeostasis in blood (Kumar and Clark, 2002)**.** Consequently, elevation of blood glucose predominantly affects RBC's, vascular endothelial cells and walls of capillaries which often leads to microvascular complications in T2DM including retinopathy, nephropathy and neuropathy (Pirart, 1978). Hyperglycaemia can lead to vascular complications through various mechanisms (D'Souza *et al.*, 2009). High blood glucose concentration or hyperglycaemia can activate several factors including nuclear factor kB, which in turn increases the expression of various genes in endothelial cells, monocyte-derived macrophages and vascular smooth-muscle cells (D'Souza *et al.*, 2009).Various mechanisms have been proposed to explain how hyperglycaemia causes vascular complications in T2DM. An increase in glucose can lead to an increase in the fluctuation of glucose to sorbitol through the polyol pathway as well as an increase in glucosamine -6-phosphate via the hexosamine pathway, activation of PKC (protein kinase C) via de novo synthesis of diacylglycerol DAG (Ahmed, 2010). The first study which reported glucose as a risk factor in the UK was the Bedford Study in 1965 and the Tecumseh Study in the U.S which suggested that elevation of glucose could be associated with CHD mortality and verified this hypothesis. The results from several clinical trials have proven that intensive glucose control can reduce the risk of microvascular complications among T2DM patients although its effect on CVD is uncertain. One of the most crucial features of T2DM is hyperglycaemia that affects

haemoglobin and membrane proteins of RBC as a result of abnormal glycation which is shown to be positively correlated with reduced membrane fluidity.

2.9.5.3.2 Full blood count:

A number of studies have reported significant associations of routine haematological parameters with T2DM and CVD. Results suggest that these abnormalities might contribute to the pathogenesis of both T2DM and CVD. In this study, the levels of various haematological parameters including haemoglobin, RBC, WBC, glucose concentration were evaluated and compared with non-diabetics. A strong association between insulin resistance syndrome and cardiovascular diseases has been demonstrated. High RBC count is a strong independent predictor of acute cardiovascular events including stroke and myocardial infarction. Haemorheological parameters including haematocrit, plasma proteins, erythrocyte aggregation, and erythrocyte deformability in T2DM patients are often disturbed. These abnormalities lead to an increase of both plasma and whole blood viscosity (WBV). Deformability of RBC‘s is one of the haemorheological parameters which is altered in T2DM patients. RBCs of T2DM patients tend to aggregate more easily when compared with healthy subjects. Excessive aggregation of RBC is one of the most important features in T2DM patients with poor glycaemic control. This has a direct effect on the WBV (Müller, 1973).

2.9.5.3.3 Lipid profiles:

A significant percentage of T2DM patients have abnormal serum lipid. Recent studies have revealed that insulin resistance is not only associated with hyperglycaemia alone, but also with several other disorders which are associated with the concentrations of lipoproteins (Grundy, 1997). In T2DM patients, typical abnormalities frequently

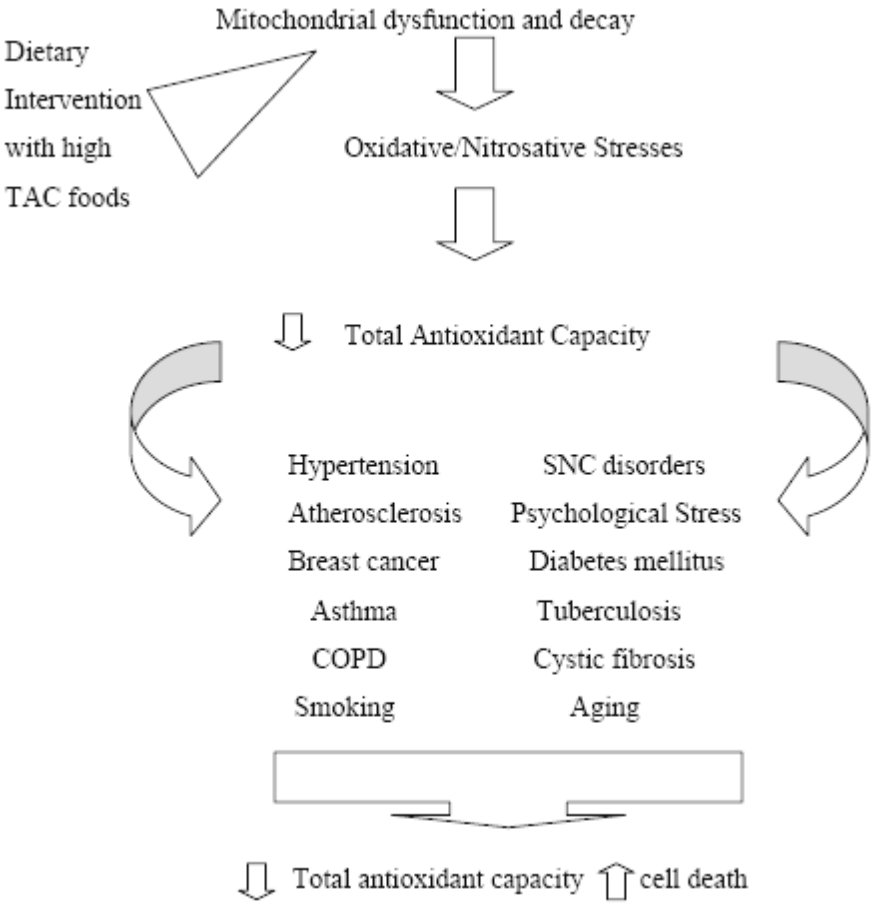
observed in lipid profile are elevated total and VLDL cholesterol, triglyceride, low levels of HDL, and a large number of dense LDL particles (Lamarche *et al.*, 1997). It is well understood that diabetic dyslipidemia is a major hallmark of metabolic syndrome and found to play an extensive role in the pathogenesis of CVD (Wannamethee *et al.*, 2007). Moreover, it has become the main reason responsible for cardiovascular morbidity and mortality in T2DM patients. An increased risk of coronary heart disease has been observed in both T2DM patients and non-T2DM subjects having triglyceridemia (Fontbonne *et al.*, 1989). The central characteristic of dyslipidemia in T2DM patients is an elevated triglyceride level, particularly triglyceride-rich VLDL and decreased HDL cholesterol levels, though concentration of LDL cholesterol does not significantly differ from non-diabetic subjects. This characteristic lipid triad is often referred to as atherogenic dyslipidemia which is commonly observed in people having premature CAD and considered as diabetic dyslipidemia when observed in T2DM patients and which leads to CVD risk. According to American Diabetes Association (ADA), increased levels of triglyceride and decreased levels of HDL is the best predictor of CVD in T2DM patients.

2.9.5.3.4 Total Antioxidant Capacity

The Total Antioxidant Capacity (TAC), a marker of oxidative stress is an assay, which summarises the levels of various antioxidants in the body. The major advantage of this test is to measure the antioxidant capacity of all antioxidants in a biological sample and not just the antioxidant capacity of a single component (Halliwell, 1994; Ferrari, 2000; Ferrari, 2001) . When antioxidant defenses are weakened, body cells and tissues become more prone to develop dysfunction and/or disease. Then, the maintenance of adequate antioxidant levels, but not overdosage is essential to prevent or even manage

greater number of disease conditions (Halliwell, 1994; Ferrari, 2000; Ferrari, 2001) . Total antioxidant capacity test, is a biomarker of disease in biochemistry, medicine, food and nutritional sciences. In many different pathophysiological conditions (heart and vascular diseases, diabetes mellitus, neurological and psychiatric disorders, renal disorders and lung diseases), TAC could be a reliable biomarker of diagnostics and prognostics, although several cautions for its use should be carefully done (choice of appropriate method, use of other antioxidant biomarkers such as cell antioxidants, genetic antioxidant-response elements (ARE) or antioxidant vitamins, and use of valuable oxidative/nitrosative biomarkers) , (Miller *et al.*, 1993). TAC could be useful to evaluate nutritional interventions with TAC-rich foods on disease risk and prevention, including anti-aging strategies. Exceptional advances in biomedical sciences since the past century gives opportunities to understand the molecular basis of disease that could result in new strategies for treatment and for prevention of pathologies, (Miller *et al.*, 1993). The involvement of reactive oxygen, nitrogen and chlorine species in disease states is strongly consolidated. Today, more than 70 pathologies are intrinsically associated with oxidative stress and its biochemical consequences, like peroxidation of lipids [measured by its markers, such as malonaldehyde, (MDA), and 4-hydroxynonenal, (4-HNE)], proteins (assessed by protein carbonyls), nucleic acids (evaluated by oxidative DNA bases) and carbohydrates (evaluated by glycosilation products) (Halliwell, 1994; Ferrari, 2000; Ferrari, 2001). Into the origin of these pathophysiologies, there is a mitochondrial dysfunction and subsequent imbalance between releasing of reactive oxygen, nitrogen or chlorine species and synthesis of defensive antioxidant capacity systems from nuclear DNA, resulting in oxidative stress. Serious consequences of the oxidative stress

include DNA damage and mutations to cell death by necrosis or apoptosis (Ferrari, 2000; Ferrari, 2001). For many decades researchers have studied many markers of oxidative stress-associated tissue damage and antioxidant defense, including measurement of antioxidant enzymes–superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx), ceruloplasmin, and proteins such as metalothionins (Ferrari, 2001). In the beginning of 1990's, (Miller *et al.*, 1993) had created a new test to measure the total antioxidant status, which has been designated as total antioxidant capacity (TAC). The major advantage of this test is to measure the antioxidant capacity of all antioxidants in a biological sample and not just the antioxidant capacity of a single compound (Halliwell, 1994; Ferrari, 2000; Ferrari, 2001).



Adapted from Carlos Kusano and Bucalen Ferrari (2008)

Figure 2.2 Pathophysiology and prevention of Total Antioxidant Capacity decay. (Arrows: stimulation, Head arrow: inhibition)

Chapter 3

MATERIALS AND METHODS

3.1 STUDY DESIGN

The study was a prospective case-control study.

3.2 STUDY SITE

The study was carried out at diabetic/hypertensive clinic and Laboratory Unit of Shai-Osudoku District Hospital, Dodowa, located in the Shai-Osudoku District of Greater Accra region, Ghana.

3.3 STUDY POPULATION

The study population consisted of 250 subjects: 100 control subjects and 150 patients withT2DM, HPTN and HPTN/T2DM as “case groups” from medical outpatient department, diabetic and hypertensive clinic of Shai-Osudoku District Hospital, Dodowa located in the Shai-Osudoku District, Greater Accra Region, Ghana.

3.4 STUDY PERIOD

The study was conducted from October 2013 to July 2015 at Shai-Osudoku District Hospital, in the Shai-Osudoku District of the Greater Accra Region of Ghana.

3.5 STUDY SUBJECTS

Based on clinical and laboratory criteria, the participants were classified into four (4) groups: normal subjects (control=100), normotensive patients with T2DM (T2DM, n=50), patients with hypertension (HPTN, n=49) and hypertensive patients with T2DM (HPTN+T2DM, n=51). HPTN was diagnosed by a consultant Physician based on

WHO-International Society of HPTN Guideline of blood pressure ≥140/90 mmHg or use of antihypertensive drugs. The diagnosis of T2DM was established based on the World Health Organisation diagnostic criteria of the fasting plasma glucose above 7.0mmol/l on at least 2 occasions or on medication for hyperglycaemia and/or 2-hour post-prandial plasma glucose above 11.1mmol/l. The fasting plasma glucose cut-point for impaired fasting glucose was set at 6.1mmol/l. The control group consisted of 100 subjects who were non diabetic and non hypertensive healthy volunteers, recruited from the Hospital.

3.6 DATA COLLECTION TOOL

A structured questionnaire was used as a tool for collection of data. The questionnaire format was modified for greater ease of understanding and clarity from a standard questionnaire. The questionnaire used in this study consisted of the following four parts:

- Socio-demographic part
- Hypertension/Diabetes type 2 and co-morbidity
- Physical activity during the past month
- Dietary habit during the past month

3.6.1 Socio-demographic part

This part elicited data about the non-modifiable factors such as age, gender, family history, as well as modifiable factors such as education, occupation etc. Questions asked in this part are quite clear and straight.

3.6.2 HPTN/T2DM and co-morbidity

This part focused on clinical characteristics; obesity, and co-morbidities such as diabetes mellitus and hypercholesterolemia. Body mass index (BMI) calculated by measuring weight (Kg) and height (m^2) was an important parameter in assessing obesity. Diabetes mellitus and hypercholesterolemia were assessed by interviewing the patients about their clinical history. Patients were given options and asked to select an answer recorded in the form of ‘’yes or no’’. It also consisted of questions regarding HPTN in terms of its diagnosis by medical persons, time or duration of diagnosis and intake of oral antihypertensive medicine.

3.6.3 Physical activity during the past month

The questions relating to physical activity focused on patients’ lifestyles. Patients were given options and asked to select an answer recorded in the form of ‘’yes or no’’ and how often the exercise was carried out.

3.6.4 Dietary habit during the past month

For this study, food was classified into fatty food and the amount of salt consumed daily. Since patients might not have been aware of, or familiar with macronutrients, they were allowed to select the food items they consumed. Frequency of fatty food intake was recorded in the form of days per week and number of times per day. Alcohol consumption behaviour was also addressed in the questionnaire. Questions was asked in the form of ‘’yes or no’’. In case of answer ‘’yes ‘’, it was asked to specify the type of alcohol they consumed, duration of consumption in terms of years.

3.7 DATA COLLECTION PROCEDURE

Written informed consent for participation was obtained from all participants and at the time of admission or entrance. Data was collected with the help of well trained health staff. Prior to data collection, the health staff were provided with training regarding appropriate techniques of interview, measurement of blood pressure, measurement of waist circumference, hip circumference, approach to the participants, and the inclusion and exclusion criteria of the study.

3.7.1 INCLUSION CRITERIA

The study population included newly and already diagnosed T2DM and hypertensive patients using WHO criteria who consented to participate in the study, the study population also included T2DM and hypertensive patients on treatment.

3.7.2 SELECTION OF CONTROL PARTICIPANTS

The control participants included males and females without T2DM and HPTN who had fasting blood glucose of less than 6.1 mmol/l and blood pressure ≤ 140/80. All members of this group underwent clinical assessment to confirm that they were free from any obvious sign of inflammation and infection.

3.7.3 EXCLUSION CRITERIA

Participants with clinical or laboratory signs of liver or renal dysfunction, malignancy, any form of inflammation, diabetes mellitus type 1, pregnant women were excluded from the study. Also patients on lipid lowering drugs or antioxidant vitamin supplements, probucol, allopurinol, quinidine, disopyramide, or other drugs known for affecting antioxidant values or failed to give a written consent were also excluded.

3.7.4 SAMPLE COLLECTION AND BIOCHEMICAL ANALYSIS

Eight (8) ml of blood was collected after an overnight fasting of about 12-16 hours, from all participants between 08:00 and 09:00 hours, local time, by venipuncture. Two (2) ml was dispensed into K_3 EDTA tube for total WBC estimation whereas two (2) ml was dispensed into fluoride oxalate tubes for plasma glucose estimation. The blood in the fluoride oxalate tubes were centrifuged at 3000rpm for 5 minutes and the plasma used for the fasting plasma glucose estimation. One (1) ml of blood was dispensed into sodium citrate tube for ESR estimation by the westergren method. Another three (3) ml was put into BD vacutainer® serum separator tubes for the estimation of different biochemical components. The samples in the serum separator vacutainer tubes were also centrifuged after 30 minutes at 3000rpm for 5 minutes at room temperature. Serum was separated into plain sample containers and frozen at –80^{o}C and later analysed for Lipid profile, Total Antioxidant Capacity, high sensitivity C - reactive protein, Tumour Necrosis factor-α. Fasting blood glucose, total cholesterol, triaglycerides were measured on an Auto-Analyser (Flexor Junior, Vital Scientific N.V.The Netherland) with reagents from ELITech group company, SEPPIM S.A.S, France. HDL-cholesterol was also analysed by Flexor Junior with reagents obtained from JAS Diagnostics, INC.USA. Total white blood cell (WBC) count was determined by Haematology Analyser, Sysmex KX-21N and Erythrocyte Sedimentation Rate (ESR) was determined manually by Westergren method. Serum High sensitivity C-reactive protein was determined by quantitative ELISA (Enzyme-Linked Immunosorbent Assay) using STAT FAX 3200 AWARENESS TECHNOLOGY-USA with reagents obtained from GenWay Biotech Inc, San Diego, USA. Tumor necrosis factor-α were determined by quantitative ELISA

using STAT FAX 3200 AWARENESS TECHNOLOGY-USA with reagents obtained from BioVision Incorporated, USA. Serum Total Antioxidant Capacity Assay kit was determined by ACE ALERA Automated Chemistry Analyser with reagents obtained from BioVision Incorporated, USA.

3.8 ASSAY PRINCIPLES

3.8.1 Total Cholesterol

The method for this assay is based on that described by Trinder, (1969). Cholesterol esterase hydrolyses esters to free cholesterol and fatty acids. The free cholesterol produced plus the preformed cholesterol is then oxidized in the presence of cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide. The quinoneimine chromogen, with absorption maximum at 500 nm, is produced when phenol is oxidatively coupled with 4-aminophenazone in the presence of peroxidase with hydrogen peroxide. The intensity of the final red colour is directly proportional to the total cholesterol concentration.

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

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

$$2H_2O_2 + Phenol + 4 - Aminoantipyrine \xrightarrow{Peroxidase} Quinoneimine + 4\ H_2O$$

Procedure

Wavelength: 500nm

Temperature: 37 °C

Read against reagent blank

	BLANK	**CALIBRATION**	**TEST**
Reagent R	300ul	300ul	300ul
Distilled Water	3ul		
Standard	-	3ul	-
Sample	-	-	3ul

Mix and read the absorbance (A) after 325 seconds incubation

CALCULATION

Sample × n standard n = standard concentration

Conversion factor: mg/dl x 0.0113 =mmol/l

mg/dl x 0.01 =g/l

3.8.2 Triglycerides

The method for this assay is based on a modified Trinder (Barham and Trinder, 1972) colour reaction to produce a fast linear endpoint reaction .Triglycerides in the sample are hydrolyzed by lipase to glycerol and fatty acids. Glycerol is then phosphorylated by adenosine-5-triphosphate (ATP) to glycerol-3-phosphate and adenosine-5-diphosphate (ADP) in a reaction catalyzed by glycerol kinase. Glycerol-3-phosphate is then converted to dihydroxyacetone phosphate (DHAP) and hydrogen peroxide

(H2O2) by glycerophosphate oxidase. The hydrogen peroxide then reacts with 4-aminoantipyrine and 3, 5 dichloro-2-hydroxybenzene (Chlorophenol) in a reaction catalyzed by peroxidase to yield a red coloured quinoneimine dye. The intensity of the colour produced is directly proportional to the concentration of triglycerides in the sample.

$$Triglycerides + H_2O \xrightarrow{Lipoprotein\ lipase} Glycerol + Fatty\ acid$$

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

$$Glycerol - 3 - Phosphate + O_2 \xrightarrow{Glycerol-3-phosphate\ oxidase} Dihydroxyacetone - P + H_2O_2$$

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

Procedure

Wavelength: 500nm

Temperature: 37 °C

Read against reagent blank

	BLANK	**CALIBRATION**	**TEST**
Reagent R	300ul	300ul	300ul
Distilled Water	3ul		
Standard	-	3ul	-
Sample	-	-	3ul

Mix and read the absorbance (A) after 425 seconds incubation

CALCULATION

Sample × n n = standard concentration
Standard

Conversion factor: mg/dl x 0.0113 =mmol/l

mg/dl x 0.01 =g/l

3.8.3 HDL-Cholesterol

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 chylomicron (CM) react with PVS and PEGME and the reaction results in inaccessibility of LDL, VLDL, and CM by cholesterol oxidase (CHOD) and cholesterol esterase (CHER).The enzymes selectively react with HDL to produce H_2O_2 which is detected through a Trinder reaction.

$$HDL + LDL + VLDL + CM \xrightarrow{PVS/PEGME} HDL + [LDL + VLDL + CM]$$

$$HDL + CHOD + CHER \xrightarrow{PVS/PEGME} Fatty\ Acid + H_2O_2$$

$$2H_2O_2 + 4 - AA + TODB \xrightarrow{Peroxidase} Quenone + 5H_2O$$

Procedure

Wavelength: 578 nm

Temperature: 37 °C

Read against reagent blank

	BLANK	**CALIBRATION**	**TEST**
Reagent R 1	240 ul	240 ul	240 ul
Distilled Water	2.4 ul		
Standard	-	2.4 ul	-
Sample	-	-	2.4 ul

Mix and after 4 min. 40 sec. of incubation, measure the absorbance (A1),then add

Reagent R2	80ul

Mix and after 4 minutes of incubation, measure the absorbance (A2).

CALCULATION

$$\frac{(A2 - A1)\ \text{sample}}{(A2 - A1)\ \text{calibrator}} \times n$$

n = standard concentration

Conversion factor: mg/dl x 0.0259 =mmol/l

mg/dl x 0.01 =g/l

3.8.4 LDL CHOLESTEROL

The LDL-Cholesterol concentration (LDL-C) is calculated from the total cholesterol concentration (TC), HDL-Cholesterol concentration (HDL-C) and the triglyceride concentration (TG) according to Friedewald equation (Friedewald *et al.*, 1972)

$$LDL(C) = TC - \left(\frac{TG}{2.2}\right) + HDL(C)$$

3.8.5 Fasting Blood Glucose

Glucose concentration in the samples was estimated with the glucose oxidase method according to the following reactions:

$$Glucose + O_2 \xrightarrow{Glucose\ oxidase} Gluconic\ Acid + 4H_2O$$

$$2H_2O_2 + Phenol + 4Aminoantiphyrine \xrightarrow{Peroxidase} Quinoneimine + 4H_2O$$

$$2H_2O_2 + 4Aminoantiphyrine\ +\ N - Ethyl - N - (\,2 - Hydroxy - 3 -$$

$$Sulfopropyl)m -\ Toluidine \xrightarrow{Peroxidase} Quinoneimine\ + 4H_2O$$

Procedure

Wavelength: 500nm

Temperature: 37 °C

Read against reagent blank

	BLANK	**CALIBRATION**	**TEST**
Reagent R	300ul	300ul	300ul
Distilled Water	3ul		
Standard	-	3ul	-
Sample	-	-	3ul

Mix and measure the variation of absorbance during 75 seconds.

CALCULATION

Sample × n n = standard concentration
Standard

Conversion factor: mg/dl x 0.0555 =mmol/l

mg/dl x 0.01 =g/l

3.8.6 Erythrocyte Sedimentation Rate (ESR) - WESTERGREN

The Erythrocyte Sedimentation Rate (ESR) is a nonspecific assay used to screen for the presence or absence of active disease. The settling of red corpuscles (red blood cells - RBCs) is due to the differential densities of the RBCs and their medium. Most often, an

increased ESR is due to an increased amount of plasma proteins (i.e., acute phase globulins and less commonly to inherent characteristics of RBCs (Wintrobe 30). ESR is measured in mm/hr using the Modified Westergren Method.

3.8.7 Total White Blood Cell Count (WBC) Using Sysmex KX-21N

This is the most popular method applied in haematology analysers manufactured not only by Sysmex but also by other companies. In this method, biological cells such as WBC, red blood cell (RBC) and platelet are regarded as completely non conductive resistivity particles. When a blood cell passes through an aperture (sensing zone) suspended in electrolyte solution, the change of electric impedance is in proportion of the volume of the cell detected and can thus separate, for e.g. RBC from platelet depending on the degree of impedance change. In the case of WBC counting, after haemolysis of RBCs, the same procedure is applied.

3.8.8 Total Antioxidant Capacity (TAC) Assay Kit

Total Antioxidant Capacity (TAC) Assay Kit is a Colorimetric assay kit bought from BioVision Incorporated Milpitas Boulevard, Milpitas, USA. Trolox is used to standardize antioxidants, with all other antioxidants being measured in Trolox equivalents. Measurement of the combined non enzymatic antioxidant capacity of biological fluids and other samples provides an indication of the overall capability to counteract reactive oxygen species (ROS), resist oxidative damage and combat oxidative stress-related diseases. In some cases, the antioxidant contribution of proteins is desired whereas in other cases only the contribution of the small molecules antioxidants is needed. BioVision developed the TAC Assay kit, which can measure either the combination of both small molecule antioxidants and proteins or small

molecules alone in the presence of our proprietary protein mask. 100*ul* Cu^{2+} working solution was added to all standard and sample wells. After 1.5 hours incubation at room temperature,Cu^{2+} ion is reconverted to Cu^{+} by both small molecule and protein. The protein mask prevents Cu^{2+} reduction by protein, enabling the analysis of only the small molecule antioxidants. The reduced Cu^{+} ion was chelated with a colorimetric probe giving a broad absorbance peak around 570nm, proportional to the total antioxidant capacity.

3.8.9 TNF-alpha (human) ELISA Kit

BioVision's Human TNF-alpha (Tumor necrosis factor-alpha) ELISA (Enzyme-Linked Immunosorbent Assay) kit bought from BioVision Incorporated Milpitas Boulevard, Milpitas, USA is an in vitro enzymelinked immunosorbent assay for the quantitative measurement of human TNF-alpha. This assay employs an antibody specific for human TNF-alpha coated on a 96-well plate. Standards and samples are pipetted into the wells and TNF-alpha present in a sample is bound to the wells by the immobilized antibody. The wells were washed and biotinylated anti-human TNF-alpha antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells were again washed, a TMB substrate solution is added to the wells and colour develops in proportion to the amount of TNF-alpha bound. The Stop Solution changes the colour from blue to yellow, and the intensity of the colour is measured at 450 nm.

3.8.10 High Sensitivity C - reactive protein (Hs-CRP) ELISA

High Sensitivity C - reactive protein (Hs-CRP) ELISA manufactured for Immuno-Biological Laboratories, Inc, Minneapolis, USA is a kit used to determine C-reactive

protein by enzyme immunoassay in human serum. The principle of the following enzyme immunoassay test follows a typical two-step capture or 'sandwich' type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for CRP is immobilized onto the microwell plate and another monoclonal antibody specific for a different region of CRP is conjugated to horse radish peroxidase (HRP). CRP from the unknown and calibrators are allowed to bind to the plate, washed, and subsequently incubated with the HRP conjugate. After a second washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microtiter plate reader. The intensity of the colour formed by the enzymatic reaction was directly proportional to the concentration of CRP in the unknown. A set of calibrators was used to plot a calibration curve from which the amount of CRP in the unknowns being tested and controls can be directly read.

3.9 ANTHROPOMETRIC VARIABLES

Height to the nearest metres without shoes and weight to the nearest 0.1 kg in light clothing were measured using a standard stadiometer (RGZ-160 Health Scale, China). The body mass index (BMI) was calculated by dividing weight (kg) by the height squared (m^2). Waist circumference (to the nearest centimetre) was measured with a Gulick II spring-loaded measuring tape (Gay Mill, WI) midway between the inferior angle of the ribs and the suprailiac crest. Hip circumference was measured as the maximal circumference over the buttocks in centimetres and the waist to hip ratio

(WHR) calculated by dividing the waist circumference (cm) by the hip circumference (cm).

3.9.1 BLOOD PRESSURE

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

3.10 CLASSIFICATION OF METABOLIC SYNDROME

3.11.1 National Cholesterol Education Program, Adult Treatment Panel III (NCEP ATP III) Criteria

One out of the three competing definitions of metabolic syndrome generally referred to in medical writings was used in the study as follows: The NCEP ATP III criteria mandate that individuals with metabolic syndrome should have three or more of the following five components of metabolic syndrome: (1) Abdominal obesity (waist circumference >102 cm for men or >88 cm for women); (2) Raised triglyceride (≥1.7 mmol L-1); (3) Low HDL-cholesterol (<0.9 mmol L-1 in men or <1.0 mmol L-1 in women); (4) High Blood Pressure (systolic BP ≥130 mmHg or diastolic BP ≥85 mmHg or treatment of hypertension) and (5) Raised fasting glucose (≥6.1mmol/l) (NCEP 2002).

3.11 STATISTICAL ANALYSIS

Results were presented as Means ± SD. The Chi-square test statistic (Fischer's exact test) was used to assess the statistical significance of categorical variables. Logistic regression test statistic was used to estimate the crude odds ratio (cOR) of age groups among the case participants. The relationships between the various parameters were assessed by spearman's rank correlation. All statistical analysis was done using a computer software program, STATA Version 11.2 for windows and data from all groups were compared using one way analysis of variance (ANOVA).A p-value < 0.05 was considered to be statistically significant.

3.12 ETHICAL CONSIDERATION

The study was ethically cleared by the Committee on Human Research, Publications and Ethics, KNUST, School of Medical Sciences and KATH, Kumasi. Written informed consent was obtained from the participants before the data collection. The participants were reassured that they could refuse to participate in the study and could withdraw at any time. The participants were free to ask any question about the study. The confidentiality of the collected data was maintained throughout the study.

Chapter 4

RESULTS

TABLE 4.1 SOCIO-DEMOGRAPHIC CHARACTERISTICS OF STUDY PARTICIPANTS

Parameter	**Cases(150)**	**Control(100)**	***P* value**	**T2DM (50)**	**T2DM & HPTN (51)**	**HPTN (49)**
Age(Years)	51.93±15.66	45.78±16.99	**0.001**	47.60±14.90	55.58±16.70***	52.71±14.53$^{\uparrow\uparrow}$
Gender						
Male, n (%)	66(44)	45(45)		25(50)	22(43.14)	19(38.78)
Female, n(%)	84(56)	55(55)	0.876	25(50)	29(56.86	30(61.22)
Educational Background						
None, n (%)	56(37.3)	15(15)		18(37.5)	16(30.8)	22(44)$^{\uparrow\uparrow}$
Basic, n (%)	52(34.7)	15(15)		18(37.5)	18(34.6)	16(32)
Secondary, n (%)	28(18.7)	20(20)	**<0.0001**	10(20.8)$^{++++}$	14(26.9)**	4(8)$^{\uparrow\uparrow\uparrow\uparrow}$
Tertiary, n (%)	14(9.3)	50(50)		2(4.2)$^{++++}$	4(7.7)****	8(16)$^{\uparrow\uparrow\uparrow\uparrow}$
Exercise						
No	120(80)	80(80)		42(84)	43(84.3)	35(71.4)
Yes	30(20)	20(20)	0.6299	8(16)	8(15.7)	14(28.6)
History Of Alcohol Intake						

None	92(61.3)	80(80)		32(64)$^{++++}$	29(56.8)****	31(63.3)$^{\uparrow\uparrow\uparrow\uparrow}$
1-5 Years	4(2.7)	5(5)		2(4)$^{++++}$	2(3.9)****	0(0)$^{\uparrow\uparrow\uparrow\uparrow}$
6-10 Years	10(6.7)	15(15)		2(4)$^{++++}$	4(7.8)****	4(8.2)$^{\uparrow\uparrow\uparrow\uparrow}$
11-15 Years	22(14.7)	0(0)	**<0.0001**	6(12)$^{++++}$	10(19.7)****	6(12.2)$^{\uparrow\uparrow\uparrow\uparrow}$
16-20 Years	16(10.6)	0(0)		7(14)$^{++++}$	3(5.9)***	6(12.2)$^{\uparrow\uparrow\uparrow\uparrow}$
>20 Years	6(4)	0(0)		1(2)	3(5.9)	2(4.1)
Family History of Disease						
Diabetes	20(13.3)	5(5)		16(32)$^{++++}$	4(7.8)	0(0)$^{\uparrow\uparrow}$
Diabetes And Hypertension	36(24)	10(10)	**<0.0001**	4(8)	21(41.2)****	11(22.5)
Hypertension	24(16)	5(5)		0(0)$^{++}$	14(27.5)****	10(20.4)$^{\uparrow\uparrow\uparrow\uparrow}$
None	70(46.7)	80(80)		30(60)$^{++++}$	12(23.5)****	28(57.1)$^{\uparrow\uparrow\uparrow\uparrow}$

Data is presented as mean (SD) or n (%) and compared using chi-square test. P value-cases compared to controls. $^{+}P< 0.05$, $^{++}P< 0.01$, $^{+++}P<0.001$, $^{++++}P<0.0001$ when the T2DM subject were compared to the control. $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P<0.001$, $^{****}P<0.0001$ when the T2DM/hypertensive subject were compared to the control. $^{\uparrow}P< 0.05$, $^{\uparrow\uparrow}P< 0.01$, $^{\uparrow\uparrow\uparrow}P<0.001$, $^{\uparrow\uparrow\uparrow\uparrow}P<0.0001$ when the hypertensive subject were compared to the control. n=Number; SD=standard Deviation. P value <0.05 were considered significant.

SOCIO-DEMOGRAPHIC CHARACTERISTICS OF STUDY PARTICIPANTS

Out of the total of 250 study participants, 100 (40%) were classified as controls and 150 (60%) were classified as cases presenting with T2DM (50), HPTN (49) and T2DM/HPTN (51). Socio-demographic characteristics of the studied population indicated that, out of the total 250 study participants, only 50 (20%) were physically active. Mean age of study participants were 49.47±16.46 years. The mean age of T2DM/hypertensive case participants (55.58±16.7 years) were significantly older than the control counterparts. There were more female participants with respect to gender distribution but with no significant difference when compared between case and control participants (p=0.8760). Few of the case participants had no education (37.3%) with an almost equal proportion with basic education (34.7%) and this was significantly higher than that of the control group (p= < 0.0001). The proportion of individuals who had attained secondary and tertiary education was significantly higher among the control group (20% and 50% respectively) than in case participants. Almost 34.7% of the study participants had a history of alcohol consumption and this was significantly higher among case participants when compared to the control group. History of T2DM, HPTN or both was higher amongst cases participants (p<0.0001) and the presence of both conditions was significantly higher in the T2DM/hypertensive participants (41.2%) as compared to the control counterparts, [Table 4.1].

TABLE 4.2 LOGISTIC REGRESSION ANALYSIS OF AGE FOR CASE PARTICIPANTS

Variable	OR(95% CI)	*P*-value
Age(Years)		
22-30	0.9 (0.4-2.3)	0.8140
31-40*	1 (0.4-2.9)	0.8630
41-50	2 (0.8-5.3)	0.1660
51-70	4.4 (1.5-12.6)	0.006

***Reference group, OR = unadjusted odds ratio and CI = confidence interval**

4.2 LOGISTIC REGRESSION ANALYSIS OF AGE FOR CASE PARTICIPANTS

Logistic regression analysis showed age was significantly associated with HPTN and T2DM and the unadjusted odds ratio (OR) for the age group (51-70 years) was 4.4, 95% CI 1.5-12.6, P=0.006. Case Participants within the age group (51-70 years) were about 4 times at risk developing T2DM or HPTN as compared to the age group 31-40 years.

TABLE 4.3 ANTHROPOMETRIC VARIABLES OF STUDY PARTICIPANTS

Parameter	Cases(150)	Control (100)	*P* value	T2DM (50)	T2DM & HPTN (51)	HPTN (49)
BMI(Kg/m^2)	27.88±4.43	23.8±4.59		28.47±4.13^{++++}	29.03±4.61****	26.08±4.03$^{\uparrow\uparrow}$
Normal Weight	46(30.7)	65(65)		19(38.0)	14(27.5)	23(46.9)
Below Normal	0(0)	5(5)		0(0)	0(0)	0(0)
Overweight	58(38.7)	20(20)		14(28.0)	14(27.5)	13(26.5)
Obese	36(24)	5(5)	**<0.0001**	13(26.0)	15(29.4)	11(22.5)
Severely Obese	10(6.6)	5(5)		4(8.0)	8(15.6)	2(4.1)
WHR	0.89±0.04	0.85±0.05		0.88±0.03^{++++}	0.89±0.02****	0.87±0.05$^{\uparrow\uparrow}$
Normal WHR	50(33.3)	80(80)		18(36)	12(23.5)	20(40.8)
Abnormal WHR	100(66.7)	20(20)	**<0.0001**	32(64)	39(76.5)	29(59.2)
SBP(mmHg)	137.3±16.7	116.5±12.01		129±13.29^{+++}	137±18.79****	139±17.65$^{\uparrow\uparrow\uparrow\uparrow}$
Normal SBP	87(58)	100(100)	**<0.0001**	44(88)	23(45.1)	20(40.8)
Above Normal SBP	63(42)	0(0)		6(12)	28(54.9)	29(59.2)
DBP(mmHg)	82±10.58	73.5±7.29		82±8.86^{++++}	82±10.65***	83±12.11$^{\uparrow\uparrow\uparrow\uparrow}$
Normal DBP	102(68)	100(100)	**<0.0001**	43(86)	31(60)	28(57)
Above Normal DBP	48(32)	0(0)		7(14)	20(40)	21(43)

Data is presented as mean (SD) or n (%) and compared using chi-square test. P value-cases compared to controls. $^{+}P< 0.05$, $^{++}P< 0.01$, $^{+++}P<0.001$, $^{++++}P<0.0001$ when the T2DM subject were compared to the control. $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P<0.001$, $^{****}P<0.0001$ when the T2DM/hypertensive subject were compared to the control. $^{\uparrow}P< 0.05$, $^{\uparrow\uparrow}P< 0.01$, $^{\uparrow\uparrow\uparrow}P<0.001$, $^{\uparrow\uparrow\uparrow\uparrow}P<0.0001$ when the hypertensive subject were compared to the control. n=Number; SD=standard Deviation. P value <0.05 were considered significant. BMI=Body Mass Index; WHR=Waist-to-hip Ratio; SBP=Systolic Blood Pressure, DBP=Diastolic Blood Pressure

ANTHROPOMETRIC VARIABLES OF STUDY PARTICIPANTS

Table 4.3 shows data on anthropometry of the study population. All indices of anthropometry (SBP,DBP,WHR and BMI) were higher ($p<0.0001$ each) in case participants compared with the control participants. SBP was controlled in about 41% of the hypertensive participants, 45% of the T2DM/hypertensive participants and as expected about 88% of the diabetic participants had normal systolic and diastolic blood pressure. The proportion of T2DM/hypertensive case participants who had higher abdominal waist-to-hip ratio was 76.5% as compared to the diabetic and hypertensive case participants who had 64% and 59% respectively. Intra-group BMI (Severely Obese) differences was observed among the cases with the proportion of severely obese patients increasing from those presenting with HPTN (4.1%) and with T2DM (8%) respectively to a maximum among the case participants presenting with both conditions (15.6%).

TABLE 4.4 ANTHROPOMETRIC VARIABLES OF STUDY PARTICIPANTS AFTER ADJUSTMENT FOR AGE

Parameter	Cases (150)	Control (100)	*P* value	T2DM (50)	T2DM & HPTN (51)	HPTN (49)
BMI (Kg/m^2)	27.68±4.39	24.00±4.77		27.64±4.12^{++++}	29.38±4.39****	25.89±3.99$^{\uparrow}$
Normal Weight	36(34)	55(61)		11(35)	11(28)	14(38.9)
Below Normal	0(0)	5(6)		0(0)	0(0)	0(0)
Overweight	36(34)	20(22)		10(32.0)	8(20.5)	18(50)
Obese	26(25)	5(6)	**<0.0001**	10(32.0)	14(35.9)	2(5.6)
Severely Obese	8(8)	5(6)		0(0)	6(15.4)	2(5.6)
WHR	0.88±0.04	0.84±0.05		0.88±0.03^{++++}	0.89±0.02****	0.87±0.05$^{\uparrow\uparrow\uparrow}$
Normal WHR	36(33.9)	75(83.3)		12(38.7)	8(20.5)	16(44.4)
Abnormal WHR	70(66.0)	15(16.7)	**<0.0001**	19(61.3)	31(79.5)	20(55.6)
SBP (mmHg)	134.5±16.40	115.6±12.10		132.3±12.1^{++++}	134.4±16.9***	136.7±17.5$^{\uparrow\uparrow\uparrow\uparrow}$
Normal SBP	50(47.2)	90(100)	**<0.0001**	13(41.9)	19(48.7)	18(50)
Above Normal SBP	56(52.8)	0(0)		18(58.1)	20(51.3)	18(50.0)
DBP (mmHg)	81±10.93	73.3±7.49		80±9.63^{++++}	80±10.51****	84±12.25$^{\uparrow\uparrow\uparrow\uparrow}$
Normal DBP	64(60.3)	90(100)	**<0.0001**	17(54.8)	27(69.2)	20(55.5)
Above Normal DBP	42(39.6)	0(0)		14(45.2)	12(30.8)	16(44.4)

Data is presented as mean (SD) or n (%) and compared using chi-square test. P value-cases compared to controls. $^{+}P< 0.05$, $^{++}P< 0.01$, $^{+++}P<0.001$, $^{++++}P<0.0001$ when the T2DM subject were compared to the control. $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P<0.001$, $^{****}P<0.0001$ when the T2DM/hypertensive subject were compared to the control. $^{\uparrow}P< 0.05$, $^{\uparrow\uparrow}P< 0.01$, $^{\uparrow\uparrow\uparrow}P<0.001$, $^{\uparrow\uparrow\uparrow\uparrow}P<0.0001$ when the hypertensive subject were compared to the control. n=Number; SD=standard Deviation. P value <0.05 were considered significant.BMI=Body Mass Index; WHR=Waist-to-hip Ratio; SBP=Systolic Blood Pressure, DBP=Diastolic Blood Pressure

4.4 ANTHROPOMETRIC VARIABLES OF STUDY PARTICIPANTS AFTER ADJUSTMENT FOR AGE

Table 4.4 shows data on anthropometry of the study population after adjustment for age. Despite the fact that age which is a confounding factor has been adjusted, all indices of anthropometry (SBP, DBP, WHR and BMI) still remained higher ($p<0.0001$ each) in case participants compared with the control participants.

TABLE 4.5 PLASMA OXIDATIVE AND INFLAMMATORY MARKERS

Parameter	Cases(150)	Controls (100)	*P* value	T2DM (50)	T2DM & HPTN (51)	HPTN (49)
TAC(mM)	1.12±0.35	1.33±0.38	0.033	1.13±0.29^{+}	1.07±0.43**	1.15±0.30$^{\uparrow}$
Hs-CRP (ng/ml)	7.31±2.05	6.53±1.42	0.011	6.95±1.90^{+}	7.93±2.67**	7.07±1.25$^{\uparrow}$
TNF-α(pg/ml)	116.46±85.74	117.60±102.30	0.8832	114.90±58.13	119.47±114.22	115.02±68.71
TotalWBC (x10^9/L)	5.79±1.84	5.68±1.33	0.1536	5.77±1.79	5.95±1.74	5.65±1.89
ESR (mmfall/hr)	35.08±24.45	17.70±9.55	<0.0001	36.16±21.92^{++++}	37.10±28.21****	31.89±22.94$^{\uparrow\uparrow\uparrow\uparrow}$

Data is presented as mean (SD) and compared using chi-square test. P value-cases compared to controls. $^{+}P< 0.05$, $^{++}P< 0.01$, $^{+++}P<0.001$, $^{++++}P<0.0001$ when the T2DM subject were compared to the control. $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P<0.001$, $^{****}P<0.0001$ when the T2DM/hypertensive subject were compared to the control. $^{\uparrow}P< 0.05$, $^{\uparrow\uparrow}P< 0.01$, $^{\uparrow\uparrow\uparrow}P<0.001$, $^{\uparrow\uparrow\uparrow\uparrow}P<0.0001$ when the hypertensive subject were compared to the control.SD=standard Deviation. P value <0.05 were considered significant.TAC=Total Antioxidant Capacity; Hs-CRP=High Sensitivity C - reactive protein; TNF-α=Tumour necrosis factor-alpha; WBC=White blood cell; ESR=Erythrocyte sedimentation rate

4.5 SERUM OXIDATIVE AND INFLAMMATORY MARKERS

Of all the inflammatory markers compared, case participants had higher levels, with significant differences in ESR ($p<0.0001$) and the Hs-CRP ($p=0.0011$) but not TNF-α ($P=0.883$) and WBC ($p=0.1536$). Total Antioxidant Capacity was decreased among case participants as compared to healthy controls ($p=0.033$). As can be seen from table 4.5, the T2DM/hypertensive case participants had lower levels of Total Antioxidant Capacity, higher levels of Hs-CRP, TNF-α and ESR as compared to the control participants. There were not much differences in measures of oxidative stress (TAC) and some inflammatory markers (Hs-CRP, TNF-α) in the individual T2DM and the hypertensive groups.

TABLE 4.6 PERCENTAGE PREVALENCE OF OXIDATIVE STRESS AND INFLAMMATION AMONG STUDY PARTICIPANTS

Parameter	Cases (150)	Controls (100)	*P* value	T2DM (50)	T2DM And HPTN (51)	HPTN (49)
TAC*(Mm)*						
Normal	143(96)	100(100)		48(96)	47(92.2)	48(97.9)
Below Normal	7(4.7)	0(0)	**0.033**	2(4)$^{+}$	4(7.8)**	1(2)$^{\uparrow}$
Hs-CRP*(ng/ml)*						
Normal	142(94.7)	100(100)		48(96)	47(92.2)	47(95.9)
Abnormal	8(5.3)	0(0)	**0.011**	2(4)$^{+}$	4(7.8)**	2(4.1)$^{\uparrow}$
TNF-α *(pg/ml)*						
Normal	150(100)	100(100)	0.8832	50(100)	51(100)	49(100)
Abnormal	0(0)	0(0)		0(0)	0(0)	0(0)
WBC *(x10^9/L)*						
Normal	130(86.7)	100(0)		44(88)	43(84.3)	43(87.8)
Abnormal	20(13.3)	0(0)	0.1536	6(12)	8(15.7)	6(12.2)
ESR *(mmfall/hr)*						
Normal	84(56)	85(85)		28(56)	25(49.1)	31(63.3)
Abnormal	66(44)	15(15)	**<0.0001**	22(44)$^{++++}$	26(50.9)****	18(36.7)$^{\uparrow\uparrow\uparrow\uparrow}$

Data is presented as figure with percentage in parenthesis and compared using chi-square test. P value-cases compared to controls. $^{+}P< 0.05$, $^{++}P< 0.01$, $^{+++}P<0.001$, $^{++++}P<0.0001$ when the T2DM subject were compared to the control. $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P<0.001$, $^{****}P<0.0001$ when the T2DM/hypertensive subject were compared to the control. $^{\uparrow}P< 0.05$, $^{\uparrow\uparrow}P< 0.01$, $^{\uparrow\uparrow\uparrow}P<0.001$, $^{\uparrow\uparrow\uparrow\uparrow}P<0.0001$ when the hypertensive subject were compared to the control.SD=standard Deviation. P value <0.05 were considered significant.TAC=Total Antioxidant Capacity; Hs-CRP=High Sensitivity C - reactive protein; TNF-α=Tumour necrosis factor-alpha; WBC=White blood cell; ESR=Erythrocyte sedimentation rate

4.6 PERCENTAGE PREVALENCE OF OXIDATIVE STRESS AND INFLAMMATION AMONG STUDY PARTICIPANTS

Table 4.6 shows percentage prevalence of oxidative stress and inflammation among the study participants. Prevalence of Oxidative Stress among the case participants was 4.7% and 0% among the control participants (p=0.033). A similar trend observed among the case participants indicates that Oxidative Stress (decreased Total Antioxidant Capacity) was significantly decreased among the T2DM (4%), T2DM/hypertensive (7.8%) and hypertensive participants (2%) as compared to the controls. Hs-CRP among the T2DM (4%) ,T2DM/hypertensive (7.8%) and the hypertensive (4.1) case participants was significantly higher as compared to the control counterparts .As can be seen from the table 4.6, WBC and ESR were high among the T2DM/hypertensive case participants. There was no significant difference with respect to serum TNF-α between cases and controls.

TABLE 4.7 BIOCHEMICAL PARAMETERS OF STUDY PARTICIPANTS

Parameter	Cases (150)	Controls (100)	P value	T2DM (50)	T2DM & HPTN (51)	HPTN (49)
Cholesterol *(mmol/l)*	5.52±1.36	4.79±0.89	**<0.0001**	5.07±1.35^{+++}	6.12±1.47****	5.35±1.04$^{\uparrow\uparrow\uparrow\uparrow}$
Triglycerides *(mmol/l)*	2.09±0.42	1.08±0.47	**<0.0001**	2.04±0.37^{++++}	2.17±0.56****	2.06±0.46$^{\uparrow\uparrow\uparrow\uparrow}$
HDL-c *(mmol/l)*	1.99±0.69	2.12±0.42	0.1071	1.95±0.65	1.92±0.69	2.12±0.72
LDL-c*(mmol/l)*	3.09±1.35	2.30±0.91	**<0.0001**	2.95±1.28^{++++}	3.37±1.50****	2.97±1.03$^{\uparrow\uparrow\uparrow\uparrow}$
VLDL-c*(mmol/l)*	0.49±0.21	0.35±0.20	**<0.0001**	0.47±0.17^{++++}	0.51±0.26****	0.48±0.21$^{\uparrow\uparrow\uparrow\uparrow}$
Coronary Risk	3.25±1.57	2.36±0.78	**<0.0001**	3.07±1.31^{++++}	3.68±1.89****	3.00±1.37$^{\uparrow\uparrow\uparrow\uparrow}$
FBG*(mmol/l)*	7.41±3.73	4.95±0.57	**<0.0001**	8.28±4.40^{++++}	7.35±2.61****	5.56±3.83$^{\uparrow\uparrow}$

Data is presented as mean (SD) and compared using chi-square test. P value-cases compared to controls. $^{+}P< 0.05$, $^{++}P< 0.01$, $^{+++}P<0.001$, $^{++++}P<0.0001$ when the T2DM subject were compared to the control. $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P<0.001$, $^{****}P<0.0001$ when the T2DM/hypertensive subject were compared to the control. $^{\uparrow}P< 0.05$, $^{\uparrow\uparrow}P< 0.01$, $^{\uparrow\uparrow\uparrow}P<0.001$, $^{\uparrow\uparrow\uparrow\uparrow}P<0.0001$ when the hypertensive subject were compared to the control. n=Number; SD=standard Deviation. P value <0.05 were considered significant. HDL-c=High Density Lipoprotein cholesterol; LDL-c=Low Density Lipoprotein cholesterol; VLDL-c= Very Low Density Lipoprotein cholesterol; FBG= Fasting Blood Glucose; Coronary Risk=total Cholesterol/HDL-c.

4.7 BIOCHEMICAL PARAMETERS OF STUDY PARTICIPANTS

As expected, fasting blood glucose levels amongst case participants were significantly higher when compared to controls. The highest mean fasting blood glucose concentration was reported among the diabetic only (8.28±4.40) followed by those with both T2DM/HPTN (7.35±2.61). Lipid indices with the exception of HDL-c assayed in this study were found to be higher amongst the case participants as compared to the control participants ($p<0.0001$ each). However, participants with T2DM/HPTN had higher levels of all these lipid profile components and this was significant for total cholesterol, triglycerides, LDL-c, VLDL-c and coronary risk ($p<0.0001$ each) but not HDL-c ($p=0.1071$).

TABLE 4.8 PREVALENCE OF METABOLIC SYNDROME AND ITS COMPONENTS AMONG STUDY POPULATION

Condition	Cases (n=150)	Control (n=100)	*P*-Value	T2D (50)	T2DM And HPTN (51)	HPTN (49)
National Cholesterol Education Programme-Adult Treatment Panel lll Criteria						
MetS	32(21.3)	2(2)	**<0.0001**	13(25.5)$^{++++}$	15(30)****	4(8.2)$^{\uparrow\uparrow\uparrow}$
WC >102,>88 (cm)	98(65.33)	15(15)	**<0.0001**	33(66)$^{++++}$	38(74.5)****	27(55.1)$^{\uparrow\uparrow\uparrow\uparrow}$
TG ≥ 1.7 (mmol/l)	16(10.67)	5(5)	0.114	4(7.8)	8(16)	4(8.16)
HDL<1.03,<1.3 (mmol/l)	16(10.67)	3(3)	**0.001**	6(12)$^{+++}$	8(15.6)***	2(4.1)
FBG ≥ 6.1 (mmol/l)	66(44)	0(0)	**<0.0001**	24(48)$^{++++}$	26(50.9)****	14(28.5)$^{\uparrow\uparrow\uparrow\uparrow}$
BP 130/85 (mmHg)	56(37.3)	0(0)	**<0.0001**	17(34)$^{++++}$	20(39.2)****	19(38.8)$^{\uparrow\uparrow\uparrow\uparrow}$

Data are presented as proportion and compared using chi-square test. P value-cases compared to controls. $^{+}P< 0.05$, $^{++}P< 0.01$, $^{+++}P<0.001$, $^{++++}P<0.0001$ when the T2DM subject were compared to the control. $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P<0.001$, $^{****}P<0.0001$ when the T2DM/hypertensive subject were compared to the control. $^{\uparrow}P< 0.05$, $^{\uparrow\uparrow}P< 0.01$, $^{\uparrow\uparrow\uparrow}P<0.001$, $^{\uparrow\uparrow\uparrow\uparrow}P<0.0001$ when the hypertensive subject were compared to the control. P value <0.05 were considered significant. MetS=Metabolic Syndrome; WC=Waist circumference; TG=Triaglycerides; HDL=High density lipoprotein; FBG=Fasting Blood Glucose; BP=Blood Pressure.

4.8 PREVALENCE OF METABOLIC SYNDROME AND ITS COMPONENTS AMONG STUDY POPULATION

The prevalence of metabolic syndrome among the case participants was significantly higher than the control participants (21.3% vs. 2%) using NCEP criteria. Irrespective of the criteria, all the components of MetS were higher among the case participants as compared to the control participants. Intra-group MetS differences was observed among the cases, with the proportion of MetS increasing from those presenting with HPTN (8.2%) and with T2DM (25.5%) to a maximum among the participants presenting with T2DM/HPTN (30%). However, all the components of MetS were higher among the T2DM/hypertensive case participants as compared to the individual T2DM and the hypertensive groups. The highest prevalence of components of MetS among the case participants was a broader waist circumference (65.33% vrs 15% in control group), high FBG (44% vrs 0% in control group) followed by raised BP (37.37 vrs 0% in control group). Triglycerides (10.67 vrs 5% in control group) and low HDL-c (10.67 vrs 3% in control group). A similar trend was observed among the 3 groups and the highest prevalence of components of MetS among the T2DM/hypertensive participants was broader waist circumference (74.5%), higher FBG (50.9%) followed by raised BP (39.2%), triglyceride (16%) and low HDL-c (15.6%).

TABLE 4.9 PERCENTAGE PREVALENCE OF CARDIOVASCULAR DISEASE RISK FACTORS AMONG THE STUDY POPULATION

Variables	Cases (150)	Control (100)	*P* value	Diabetes type 2 (50)	Diabetes type 2 & Hypertension (51)	Hypertension (49)
Hypercholesterolaemia	62(41.3)	25(25)	**<0.0001**	21(42)$^{++++}$	23(45)****	18(36.7)$^{\uparrow\uparrow\uparrow}$
Hypertriaglyceridaemia	14(9.3)	5(5)	0.205	5(10)	6(11.7)	4(8.2)
Low HDL	36(24)	3(3)	**0.001**	14(28)$^{+++}$	15(29.4)***	7(14.3)$^{\uparrow\uparrow}$
High LDL	26(17.3)	8(8)	**<0.0001**	7(14)	13(25.4)****	6(12.2)
Coronary Risk ≥ 5	16(10.67)	3(3)	**0.001**	4(8)$^{+}$	10(19.6)****	2(4.1)
Physical Inactivity	120(80)	80(80)	0.6299	42(84)	43(84.3)	35(71.4)
Obesity(BMI)	46(30.7)	10(10)	**<0.0001**	17(34)$^{++++}$	23(45.1)****	13(26.5)$^{\uparrow\uparrow\uparrow\uparrow}$
Obesity-WHR	110(66.7)	20(20)	**<0.0001**	32(64)$^{++++}$	39(76.5)****	29(59.2)$^{\uparrow\uparrow\uparrow\uparrow}$
Obesity-WC	98(65.3)	31(31)	**<0.0001**	33(66)$^{++}$	38(74.5)****	27(55.1)$^{\uparrow\uparrow}$

Data are presented as proportion and compared using chi-square test. P value-cases compared to controls. $^{+}P< 0.05$, $^{++}P< 0.01$, $^{+++}P<0.001$, $^{++++}P<0.0001$ when the T2DM subject were compared to the control. $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P<0.001$, $^{****}P<0.0001$ when the T2DM/hypertensive subject were compared to the control. $^{\uparrow}P< 0.05$, $^{\uparrow\uparrow}P< 0.01$, $^{\uparrow\uparrow\uparrow}P<0.001$, $^{\uparrow\uparrow\uparrow\uparrow}P<0.0001$ when the hypertensive subject were compared to the control. P value <0.05 were considered significant. HDL-High density lipoprotein ,LDL-Low density lipoprotein ,BMI-Body mass index, WHR-Waist-to-hip ratio, WC-Waist circumference

4.9 PERCENTAGE PREVALENCE OF CARDIOVASCULAR DISEASE RISK FACTORS AMONG THE STUDY POPULATION

Table 4.9 shows data on percentage prevalence of cardiovascular risk factors among the study population. The highest prevalence of cardiovascular risk factor among the case participants is physical inactivity (80%) followed by abdominal obesity as measured by WHR (66.7%), then central obesity as measured by WC (65.3%) and hypercholesterolaemia (41.3%).The least cardiovascular risk factor among the case participants was hypertriaglyceridaemia (9.3%). The highest prevalence of cardiovascular risk factor among the control participants is physical inactivity (80%), central obesity as measured by WC (31%), then hypercholesterolaemia (25%). The least among them is low HDL-c (3%). The prevalence of hypercholesterolaemia (41.3%), Low HDL-c (24%), high LDL-c(17.3%),coronary risk (10.67%),obesity BMI (30.6%), Obesity-WHR (66.7%) and Obesity-WC (65.3%) among the case participants were significantly higher as compared to the control participants (25%,3%,8%,3%,10%,20% and 31%) respectively using chi-square test of significance. It is noteworthy that apart from the physical inactivity which was high in the 3 groups of case participants, the next highest cardiovascular risk factor among the T2DM/hypertensive and the hypertensive participants was abdominal obesity as measured by WHR (76.5% and 59.2%) respectively. In the diabetic case participants, central obesity, WC (66%) is next to physical inactivity. It is noteworthy that central obesity, WC (74.5%), abdominal obesity, WHR (76.5%), general obesity, BMI (45.1%), Coronary risk (19.6%) and high LDL-c (25.4%) among the T2DM/hypertensive case participants were significantly higher than the control participants.

TABLE 4.10 SPEARMAN'S (RHO) CORRELATION BETWEEN DEMOGRAPHIC, INFLAMMATORY, ANTHROPOMETRIC AND SOME BIOCHEMICAL VARIABLES FOR CONTROLS

	SBP	AGE	WC	WHR	BMI	CR	TAC	FBG	Hs-CRP	TNF-α
SBP	1.0000									
AGE	0.4857****	1.0000								
WC	0.0356	0.0877	1.0000							
WHR	0.3849***	0.3705***	0.4388****	1.0000						
BMI	0.1666	0.0084	0.7708****	0.3451**	1.0000					
CR	0.1853	-0.0754	0.2060*	0.3929***	0.3286	1.0000				
TAC	-0.2318*	0.0084	0.0241	-0.1137	0.0870	-0.1846	1.0000			
FBG	-0.1592	-0.0577	0.1660	-0.0708	0.0225	0.2173*	-0.5883****	1.0000		
Hs-CRP	0.1177	0.2959**	0.2284*	0.2150	0.2492*	0.0943	0.0577	0.1979*	1.0000	
TNF-α	-0.1089	-0.1126	0.1932	0.0218	-0.0124	0.3085**	0.0468	0.1107	0.0844	1.0000

*SBP=Systolic Blood Pressure,WC=Waist Circumference,WHR=waist-to-hip ratio,BMI=Body mass index,CR=Coronary risk,FBG=Fasting blood glucose,Hs-CRP=High sensitivity C-reactive protein,TNF-α=Tumour necrosis factor-alpha.*Correlation is significant at <0.05,** Correlation is significant at <0.01,***Correlation is significant at <0.001.****Correlation is significant at <0.0001*

4.10 SPEARMAN'S (RHO) CORRELATION BETWEEN DEMOGRAPHIC, INFLAMMATORY, ANTHROPOMETRIC AND SOME BIOCHEMICAL VARIABLES FOR CONTROLS

A spearman's correlation for association between selected variables amongst controls is shown in table 4.10. Age was significantly and positively associated with High Sensitivity C-Reactive Protein (Hs-CRP) and Waist-to-hip ratio (WHR). There was a positive significant relation between Hs-CRP, Fasting Blood Glucose (FBG) and Body Mass Index (BMI). Waist Circumference (WC) also showed a positive significant relation with WHR, BMI and Hs-CRP. The Total Antioxidant Capacity (TAC) showed a significant negative relation with FBG, WHR and SBP. There was a positive significant association between Coronary risk (CR), FGB and Tumour Necrosis Factor-α (TNF-α).

TABLE 4.11 SPEARMAN'S (RHO) CORRELATION OF SOME BIOCHEMICAL VARIABLES FOR CASE PARTICIPANTS

	SBP	AGE	TAC	FBG	Hs-CRP	TNF-α	ESR	WBC
SBP	1.0000							
AGE	0.3431****	1.0000						
TAC	-0.0873	0.0544	1.0000					
FBG	0.2974****	0.0742	-0.1329*	1.0000				
Hs-CRP	0.2288***	0.0377	0.1735**	0.1182	1.0000			
TNF-α	0.0074	-0.1126	0.0336	-0.1038	0.0844	1.0000		
ESR	0.2673****	0.2804	0.3292***	0.0768	0.2553*	-0.3164**	1.0000	
WBC	-0.1224	-0.0498	-0.1021	0.0452	0.2097*	0.12777	0.2804**	1.0000

*SBP=Systolic Blood Pressure, FBG=Fasting blood glucose, Hs-CRP=High sensitivity C- reactive protein,*Correlation is significant at <0.05, ** Correlation is significant at <0.01, ***Correlation is significant at <0.001. ****Correlation is significant at <0.0001*

4.11 SPEARMAN'S (RHO) CORRELATION OF SOME BIOCHEMICAL VARIABLES FOR CASE PARTICIPANTS

From the table above, hs-CRP showed a positive significant relation with Erythrocyte Sedimentation Rate (ESR) and White Blood Cells (WBC). TAC also showed a positive significant relation with Hs-CRP, ESR and negatively with WBC and FBG. Systolic Blood Pressure (SBP) was positively and significantly associated with Age, FBG, Hs-CRP and ESR..TAC showed a negative correlation with SBP but did not reach a level of significance. There was a positive significant association between ESR and WBC.

TABLE 4.12 SPEARMAN'S (RHO) CORRELATION OF SOME BIOCHEMICAL VARIABLES FOR CASE PARTICIPANTS

	SBP	Hs-CRP	FBG	CHOL	TRIG	HDL-c	LDL-c	WC	CR	TAC
SBP	1.0000									
Hs-CRP	0.7282****	1.0000								
FBG	0.2974****	0.1182	1.0000							
CHOL	-0.0893	-0.158	0.0275	1.0000						
TRIG	0.2363	0.2182***	0.2698****	0.1380	1.0000					
HDL-c	-0.2363	-0.0910	-0.3002****	-0.0590	-0.4683****	1.0000				
LDL-c	0.2877****	0.0844	0.1236	0.859****	0.2789****	-0.2949****	1.0000			
WC	0.3453****	0.1328*	0.2084***	0.2140**	0.3198****	-0.2738****	0.2183***	1.0000		
CR	0.3435****	0.1182	0.3314****	0.397***	0.5406****	-0.7651****	0.6690****	0.3319****	1.0000	
TAC	-0.0873	0.1735**	-0.1329*	0.0758	-0.0044	-0.0898	0.0447	-0.0029	0.1043	1.0000

*SBP=Systolic Blood Pressure, BP=Diastolic Blood Pressure, CHOL=Cholesterol, TRIG=Triaglycerides, HDL-c=High Density Lipoprotein, LDL-c=Low Density Lipoprotein, WC=Waist Circumferenc, CR=Coronary risk, FBG=Fasting blood glucose.*Correlation is significant at <0.05, ** Correlation is significant at <0.01, ***Correlation is significant at <0.001. ****Correlation is significant at <0.0001*

4.12 SPEARMAN'S (RHO) CORRELATION OF SOME BIOCHEMICAL VARIABLES FOR CASE PARTICIPANTS

Table 4.12 shows spearman's correlation of some selected biochemical variables of case participants. Amongst the cases, SBP showed a significant and a positive correlation with Hs-CRP, LDL-c, WC, Coronary risk (CR) and negatively with High Density Lipoprotein (HDL-c). A significant positive relation was observed with FBG, Triglycerides, WC and CR. Hs-CRP also showed a significant positive relation with Triglycerides, WC and TAC. Total Cholesterol showed a significant positive correlation with LDL-c, WC and CR .Another positive significant relation was observed between triglycerides, LDL-c, WC, CR and negatively with TAC. LDL-c showed a significant positive correlation with WC and CR. TAC also showed a significant negative relation with FBG.

TABLE 4.13 SPEARMAN'S (RHO) CORRELATION OF ANTHROPOMETRY AND BIOCHEMICAL VARIABLES FOR CASE PARTICIPANTS

	AGE	SBP	WC	BMI	WHR	FBG
AGE	1.0000	0.3431****	0.1386*	0.1208	0.1680*	0.0742
SBP		1.0000	0.3453****	-0.0477**	0.2280**	0.2934****
WC			1.000	0.7572****	0.4388****	0.2084***
BMI				1.000	0.152	0.1056
WHR					1.0000	0.110
FBG						1.0000

*SBP=Systolic Blood Pressure, FBG=Fasting blood glucose, WC=Waist circumference, BMI=Body Mass Index, WHR=Waist/Hip circumference ratio,*Correlation is significant at <0.05, ** Correlation is significant at <0.01, ***Correlation is significant at <0.001. ****Correlation is significant at <0.0001*

4.13 SPEARMAN'S (RHO) CORRELATION OF ANTHROPOMETRY AND BIOCHEMICAL VARIABLES FOR CASE PARTICIPANTS

From the table above, SBP showed a significant positive correlation with age, WC, WHR , FBG and negatively with BMI. There was also a significant positive correlation between FBG and WC. Age also showed a significant positive correlation with WC and WHR. There was also a positive correlation between Age and FBG but did not reach a level of significance.

Chapter 5

DISCUSSION

5.1 The relationship between Age, Hypertension and Type 2 Diabetes

Results from this study indicated that the prevalence of HPTN and T2DM increased with increasing age. From Table 4.2, logistic regression analysis showed that age category 51-70 years was significantly associated with HPTN and T2DM ; unadjusted odd ratio was 4.4, 95% CI 1.5-12.6, P=0.006. This means that case participants within the age group (51-70 years) were about 4 times at risk of developing T2DM and HPTN compared to the age group 31-40 years. The finding is consistent with those reported earlier in Ibadan-Nigeria (Akinkugbe, 1969), Bursa-Turkey (Aksu *et al.*, 2006), Benin-Nigeria (Idemudia and Ugwuja, 2009) and Rajahmundry-India (Kumar *et al.*, 2010) who observed a positive relationship between age and HPTN as well as T2DM. A study in Tanzania also revealed that the prevalence of hypertension was higher after the age of 50 years (Bovet *et al.*, 2002). Although not well understood, the mechanism underlying increased blood pressure with age could be attributed to the effects of environmental factors such as sensitivity to salt intake, increased BMI, and alcohol consumption (Kornitzer *et al.*, 1999). These environmental factors may cause the blood pressure to rise due to the reduced elasticity of the arterial system; a process which may not progress uniformly at all sites of the arterial system (Gillessen *et al.*, 1995). An increased incidence of type 2 diabetes is also observed with age, and there are many possible reasons for this occurrence. One of these is that the beta cell has reduced proliferative capacity and in diabetic individuals this is further

confounded by higher rates of beta cell apoptosis. The currently known underlying mechanisms behind the reduction in beta cell proliferation observed with age include reduced expression of cell cycle activators, increased expression of cell cycle inhibitors, reduced *pdx1(pancreatic and duodenal homeobox 1)* expression, and increased amylin aggregation (Scheen, 2005). This implies that, the older we get, the greater the risk for developing high blood pressure; also a risk factor for developing T2DM.

5.2 Role of Oxidative Stress in Hypertension and Type 2 Diabetes

5.2.1 Serum Total Antioxidant Capacity

Kusano and Ferrari, (Kusano and Ferrari, 2008), focused on recent applications of the total antioxidant capacity (TAC) test in medical and nutritional studies as well as future possible uses of TAC as a biomarker for diagnosis, prognosis and prevention of diseases. In type 2 diabetic patients, TAC was significantly decreased, this decrease was more appreciable in participants presenting with both T2DM and HPTN with mean value of $1.07\pm0.43mM$ (Table 4.5). Total antioxidant capacity was significantly negatively correlated with fasting blood glucose ($p<0.05$) among the case participants. Studies of Mahmood and Al Neaimy (Mahmood and Al Neaimy, 2008) and (Đorđević *et al.*, 2008) in Serbia showed a low TAC level among patients presenting with type 2 diabetes compared to their healthy control peers. However, (Savu *et al.*, 2012), observed a higher serum TAC levels in uncomplicated type 2 diabetic patients compared to control subjects among Romanians. The difference in population characteristics may well be the explanation for the varied measures of oxidative stress levels recorded among persons with glycaemic dysregulation. In

accordance to our study, (Bovet *et al.*, 2002) reported lower TAC level in both complicated and uncomplicated type 2 diabetic patients compared to healthy group. Moreover, recent studies conducted by (Gul *et al.*, 2010) and (Bertin *et al.*, 2000) showed that TAC is significantly decreased in diabetic type 2 patients, and also in patients with macrovascular complication (Opara *et al.*, 1999; Aslan *et al.*, 2007). The reduced total antioxidant defense mechanism observed in this study agrees with findings of (Brownlee, 2005; Jay *et al.*, 2006; Uzar *et al.*, 2012), who explained the decrease could be due to increased oxidative stress and free radical formation in diabetes mellitus. Hyperglycaemia-induced oxidative stress directly promotes endothelial dysfunction via several mechanisms including glucose autoxidation, the formation of advanced glycated end products (AGEs) and activation of the polyol pathway (Jay *et al.*, 2006). Recently, a unified mechanism has been proposed by (Brownlee, 2005) to explain the role of hyperglycaemia-induced oxidative stress in diabetic complications. Upon hyperglycaemia, there is an increase in electrons entering the mitochondrial electron transport chain during glucose oxidation. The voltage gradient across the mitochondrial membrane will therefore rise beyond a certain threshold so that the electron transfer is blocked inside complex III of the electron transport chain. Electron leaking then occurs at the position previous to complex III (i.e. Coenzyme Q) to generate superoxide. Consequently, over production of superoxide from the mitochondrial electron transport chain inhibits the activity of the key glycolytic enzyme glyceraldehydes-3 phosphate dehydrogenase (GAPDH). Hyperglycaemia is reported to deplete antioxidants and to increase oxidative stress, and

this may be central to the development of vascular complications in diabetes (Baynes and Thorpe, 1999; Laight *et al.*, 2000). Total antioxidant capacity is significantly negatively correlated with Fasting blood glucose (p <0.05). These interesting correlations indicate that decreased antioxidant capacity or defense plays an important role in the pathogenesis of complications in patients with T2DM. The results obtained from this present study showed a significantly *(p < 0.05)* lower TAC values for hypertensive compared with normotensive. It also showed a significantly (*$p<0.01$*) decreased TAC values for T2DM/hypertensive participants as compared to the controls. In the present study,7.8% of T2DM/hypertensive patients had low levels of TAC as compared to the hypertensive (2%). This is in agreement with the findings of Adegor,2010 that serum TAC is decreased in T2DM and HPTN (Adegor, 2010). In this study, plasma levels of TAC showed a significant negative correlation with SBP among the control participants (p <0.01). Another finding in this study was that there was a negative correlation between TAC and SBP among the case participants but did not reach a level of significance. Despite the non-significant correlation differences between TAC and SBP among the 3 groups, compared to the control participants, the mean for the T2DM, T2DM/Hypertensive groups (Table 4.5), suggest that the T2DM/Hypertensive patients present a high risk for cardiovascular development (mean value,1.07±0.43) and T2DM patients present an intermediate risk for cardiovascular disease (mean value, 1.13±0.29) as a result of low plasma levels of TAC. The observed reduction in TAC among the hypertensive could be due to the presence of high amount of free radicals and other oxygen derived (superoxide) species. This is in line with findings of (Adegor, 2010) who investigated the levels of serum total antioxidants

among hypertensive in Abraka, Delta State, Nigeria. In their study, it was revealed that HPTN significantly reduced total antioxidant level in both gender. Shortlived free superoxide and nitric oxide have been shown to react chemically to form highly reactive free radicals such as peroxynitrite that triggers the depletion of plasma antioxidants and increased lipid peroxidation (Padayatty *et al.*, 2003). The effects of decreased TAC in HPTN are enormous as the product of lipid peroxidation such as F2-isoprostanes reacts chemically to activate inflammatory immune response and complicate coronary heart disease including HPTN (Adegor, 2010). HPTN/T2DM patients were observed to have lower levels of TAC, followed by T2DM and the hypertensive. This finding suggests that patients with the 2 associated diseases have a more active oxidative stress.

5.3 Role of Inflammation in Type 2 Diabetes and Hypertension

Type 2 diabetes mellitus (DM_2) and high blood pressure (HBP) may contribute to the development of cardiovascular disease and inflammation may be an important factor in these conditions (Lyra *et al.*, 2006). The present study reveals that serum high sensitivity- C-reactive protein (hs-CRP) levels showed a significant, positive correlation ($p<0.001$) with systolic blood pressure among the case participants. Our findings are in agreement to that one reported by (Lakoski *et al.*, 2005) who also have shown a positive link between elevated CRP and systolic blood pressure in the Women's Health study cohort. There are several potential mechanisms that may account for the observed relationship between blood pressure and hs-CRP levels. HPTN may lead to multiple inflammatory stimuli at the vessel wall which in turn promote the production of a

number of pro inflammatory cytokines such as CRP, interleukin-6 and TNF-α as a defense against injurious factors (Yoshizumi *et al.*, 1993; Zietz *et al.*, 1999; Bautista *et al.*, 2005). Moreover, high levels of CRP may upregulate angiotensis receptors and enhance expression of plasminogen activator inhibitor-1 by endothelial cells and these changes could raise blood pressure and promote atherogenesis (Sattar *et al.*, 2003; Savoia and Schiffrin, 2006). There was a positive correlation between hs-CRP and FBG among the case participants but the association did not reach a level of significance. This could mean that inflammation affects diabetes mellitus in pathways that are independent of each other. This is in line with findings of (Lyra *et al.*, 2006) who showed an independent association between hs-CRP and FBG among Portuguese T2DM patients. Significantly greater number of participants presenting with both chronic conditions (7.8%) than T2DM (4.0%) and HPTN (4.1%) exhibited abnormal serum hs-CRP levels. The finding suggests a concomitant action of these two disease states (T2DM and HPTN) in the influence of increased inflammatory process that is reflected by an increase in CRP levels. Pro-inflammatory process exacerbation appears to be the mechanism by which high glucose levels in association with high blood pressure increases the risk of cardiovascular disease. (Pitocco *et al.*, 2010) suggested that increased glucose levels lead to increased mitochondrial formation of reactive oxygen species (ROS), superoxide, producing peroxynitrite after reacting with nitric oxide; this process induces cellular damage through depletion of the co-factor of the endothelial isoform of nitric oxide synthase (eNOS), tetrahydrobiopterin (BH4). The pathway of peroxynitrite-mediated injury involves DNA strand breakage, activation of the nuclear enzyme poly (ADP-ribose) polymerase (PARP), and inhibition of

glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Pitocco *et al.,* 2010). It also activates the "classic" pathways of diabetic complications, including: i) the polyol pathway, ii) the advanced glycation end products (AGE) pathway, iii) the protein kinase C (PKC) pathway, and iv) the hexosamine pathway (Pitocco *et al.*, 2010). PARP activation can also up regulate various proinflammatory pathways, which lead to pathological modifications in adhesion molecule expression, angiogenesis, and other processes (Pitocco *et al.*, 2010). Inflammation is one manifestation of oxidative stress and the pathways that generate the mediators of inflammation, such as adhesion molecules and interleukins, are all induced by oxidative stress (Vîrgolici *et al.*, 2008). We could not find a significant difference in the levels of serum TNF-α between case and control participants in the present study. Our results compare with (Zietz *et al.*, 1999) who did not observe any significant difference in the levels of TNF-α between case and control participants. It is believed that adiposity is the primary factor underlying the observed result (Bertin *et al.,* 2000). Adipose tissue is an important mediator of oxidative stress and inflammation, contributing to the production of reactive oxygen species and pro-inflammatory cytokines, including TNF-**α** (Mohamed-Ali *et al.*, 1998). Moreover, expression and secretion of these inflammatory mediators increase in proportion to adiposity (Kern *et al.,* 2001) and are largely determined by body fat distribution, for example excess visceral fat accumulation, not subcutaneous fat, is associated with greater production of TNF-α (Bertin *et al.,* 2000)**.** However, studies conducted in USA has demonstrated that serum levels of TNF-α were significantly higher in patients with T2DM and HPTN (Bautista *et al.*, 2005; Mirza *et al.*, 2012) than the control participants. The mechanism behind this finding is not well

understood but it is believed that TNF-α decreases endothelial nitric oxide synthase mRNA level by shortening its half-life (Vidyasagar *et al.*, 2013). This may result in decreased bioavailability of nitric oxide and lead to endothelial dysfunction, chronic vasoconstriction, insulin resistance and elevated blood pressure (Vidyasagar *et al.*, 2013).

5.4 The Role of Metabolic Syndrome and Inflammation in Hypertension and Type 2 Diabetes

The metabolic syndrome is the concurrence of hyperglycaemia, mild dyslipidaemia, HPTN and visceral obesity that substantially increases the risk of developing cardiovascular diseases and T2DM (Alexander *et al.*, 2003). In the current study, we evaluated the relationship of hs-CRP with the components of metabolic syndrome in both control and case participants. The metabolic syndrome was diagnosed using modified NCEP ATP III criteria. The San Antonio Heart Study suggested that although both definitions (WHO and NCEP-ATP III) were predictive in the general population, the simple NCEP-ATP III definition tended to be more predictive in lower-risk subjects (Hunt *et al.*, 2004). Hence we used the modified NCEP ATP III criteria for our study. Associations between hs-CRP and the components of a lipoprotein-lipid profile have been identified in literature (Lemieux *et al.*, 2001), yet TG was the only component with a significant relationship ($p=0.0005$) in the current study. This confirms studies conducted by (Lemieux *et al.*, 2001) who demonstrated a significant positive relationship between Hs-CRP and TG among a cohort of adult men presenting with metabolic syndrome. The significant positive relationship between CRP and TG

values may suggest that there is a link between inflammation and the body's ability to clear fat from the blood. Cytokines such as IL-6 and TNF-a stimulate CRP production in the liver and these cytokines also affect the activity of lipoprotein lipase, which is the protein that is responsible for the breakdown of lipoproteins (Lemieux *et al.*, 2001). In this study, there was a significant positive correlation between high blood pressure (component of metabolic syndrome) and hs-CRP (p=0.0003). (Schillaci and Pirro, 2006) have shown a positive correlation between high blood pressure and hs-CRP in an earlier study. Though the understanding of the relationship between blood pressure and inflammation is still receiving much attention, Schillaci and Pirro (2006) suggested that C-reactive protein is involved in complex pathways leading to endothelial dysfunction, increased peripheral vascular resistance and large artery stiffness in hypertension. There was a significant positive correlation between hs-CRP and waist circumference (WC) (p=0.0359). Among men and women with type 2 diabetes mellitus from the ATTIC

A study, (Pitsavos *et al.*, 2007) revealed a positive correlation between hs-CRP and central obesity consistent with the results of this study. A possible mechanism explaining this link is that adipocytes in obese patients with metabolic syndrome release into the circulation high amounts of TNF-α and IL-6 into the circulation (Pitsavos *et al.*, 2007), which stimulates the production of CRP by the liver and induce insulin resistance. Insulin resistance itself is responsible for the higher level of cytokine production (Mørkrid *et al.*, 2010). Hence one of the important determinants of the systemic chronic low grade inflammation is probably central obesity.

5.5 SERUM LIPIDS, HYPERTENSION AND DIABETES TYPE 2

Lipid metabolic abnormalities play an important role in various diseases such as hyperlipidemia, hypertension and diabetes mellitus (Felts and Rudel, 1975; Gurr, 1975;

Liebich, 1986). In the present study, it has been found that the mean cholesterol and triglyceride levels were significantly higher in all case participants compared to healthy controls; these higher level were more marked in patients with both T2DM and HPTN (Table 4.9). The finding is consistent with the study of (Lupachyk *et al.*, 2012) who found higher triglyceride and cholesterol level in T2DM/hypertensive participants. In their view, (Okoduwa *et al.*, 2013) suggested that hyperlipidemia is instrumental in the progression of complications of diabetes and hypertension. Our study revealed combined hypercholesterolaemia, low high-density lipoproteinaemia and hypertriglyceridaemia among case participants when compared to the controls. .The cause of lipid alterations among persons presenting with T2DM and HPTN case have been attributed to differential insulin distribution which leads to very low density lipoprotein cholesterol (VLDL-C) and triacylglycerol production through hepatic hyperinsulinaemia accompanied by decreased catabolism of triacylglycerol-rich lipoprotein as a result of relative peripheral insulin deficiency (Reaven, 1987). Hypertriglyceridemia and hypercholesterolemia are associated with oxidative modification of LDL-C, protein glycation and glucose auto oxidation, thus leading to excess production of lipid peroxidation products which may cause elevation of oxidative stress in higher lipid and hyperlipidemic subjects (Lakoski *et al.*, 2005).

5.6 Obesity in Type 2 Diabetes and Hypertension

Studies have shown that both T2DM and HPTN are strongly associated with increased insulin resistance and obesity, besides being powerful risk factors for CVD (Preik *et al.*, 2000; Natali *et al.*, 2006). Our results have confirmed these assertions. After adjustment

for age, measures of obesity were found to be strongly associated with glycaemia and systolic blood pressure. This study therefore used WC, WHR alongside BMI as has been recognised as a substantial component in the assessment of cardiovascular disease risk factors due to its strong positive association with HPTN and T2DM (Porth and Kunert, 2002; Dalton *et al.*, 2003; Shahbazpour, 2003). Among measures of obesity and adiposity, WC remains most significant when the case participants were compared to the controls. Measures of central obesity provide additional predictive information for the risk of disease, especially T2DM and HPTN, beyond that which is provided by measures of overall obesity. This is especially the case among those in the upper extremes of central obesity distributions (Chan *et al.*, 1994). Waist circumference (WC) is an excellent indicator of abdominal adiposity (Ross *et al.*, 1992). It is also strongly related to abdominal subcutaneous fat, total abdominal fat as well as total body fat (Lean *et al.*, 1996). It is used in metabolic syndrome definitions and diabetes risk scores as a marker of central obesity (Parikh *et al.*, 2007). In this study, there was a strong significant positive correlation between SBP and WC ($p=<0.00001$). There was also another strong significant, positive correlation between WC and FBG ($p=<0.0001$). This study demonstrated that a greater number of the diabetic type 2 and hypertensive participants had high level of abdominal adiposity which put them at high risk of developing cardiovascular disease. The findings of this study confirmed that T2DM and HPTN are associated with obesity. This is in agreement with the findings of (Griesmacher *et al.*, 1995) that, obesity BMI,WC is high in T2DM and HPTN**.** Also, the higher BMI,WC in these diseases could be attributed to increased calorie intake which leads to obesity as observed in the T2DM, hypertensive and T2DM/

hypertensive groups (Khanna *et al.*, 2008). It could also be due to sedentary life style, resulting from decreased exercises as the individuals increase in age (Okoduwa *et al.*, 2013). In this study, there was a significant, negative correlation between TAC, BMI/ WC. The results indicate that the antioxidant status of the study groups was impaired, as compared to the controls. Obesity is a pathological condition which is spawned by excess adiposity (Keaney *et al.*, 2003). Obesity is closely associated with oxidative stress. The possible mechanism of obesity related oxidative stress includes increased oxygen consumption and subsequent free radical production via mitochondrial respiration, diminished antioxidant capacity, increased fat deposition and cell injury, thus causing increased rates of free radical formation (Brown *et al.*, 2009).

Chapter 6

CONCLUSION

6.1 CONCLUSION

- The study demonstrated that age is positively associated with HPTN and T2DM; unadjusted odd ratio was 4.4, 95% CI (1.5-12.6), (P=0.006). This means that the case participants within the age group (51-70 years) were about 4 times at risk developing T2DM and HPTN compared to the age group (31-40 years).
- The results indicate that the antioxidant capacity of the study groups was significantly decreased, as compared to the controls and was more prominent in participants presenting with both T2DM and hypertension. This could play a key role in predisposing the diseased participants to future cardiovascular disease.
- The case participants exhibited significantly higher levels of inflammation as measured by hs-CRP and ESR with the exception of TNF-α and WBC levels.
- Metabolic syndrome and its components were significantly found to be more prevalent and positively associated with inflammation among patients presenting with HPTN and T2DM than the control group. These unfavorable risk factor variables could be playing key roles in predisposing the diseased participants to future cardiovascular disease.
- Both T2DM and HPTN are strongly associated with obesity, besides being powerful risk factors for cardiovascular disease. A greater number of T2DM and hypertensive case participants had hyperlipidemia, high level of abdominal adiposity which put them at high risk of developing cardiovascular disease.

6.2 RECOMMENDATION

- Since inflammation is known to affect T2DM and HPTN in pathways that implicate endothelial dysfunction, hence endothelial markers of vascular integrity such as VCAM-1, Oxidized LDL and Nitric Oxide in the various disease groups can be investigated.
- The scope of inflammatory markers could be extended to include IL-6 since it is suggested to be strongly associated with T2DM and HPTN.
- The sample size could also be expanded in order that findings could be generalized.
- Since the antioxidant status of the study groups was impaired, fruit and vegetable consumption should be encouraged to boost the antioxidant level of type 2 diabetics and the hypertensive patients.
- TAC and hs-CRP can be added to the routine test to measure oxidative stress and inflammation among T2DM and the hypertensive patients to minimize their risk of developing cardiovascular disease.

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APPENDIX

Participant Information Leaflet and Consent Form

<u>This leaflet is given to all prospective participants to enable them know enough about the research before deciding to or not to participate</u>

Title of Research:

Oxidative Stress and Inflammation in patients presenting with hypertension and type 2 diabetes at the Shai-Osudoku District Hospital, Dodowa.

Name(s) and affiliation(s) of researcher(s):

This study was being conducted by Dr. W.K.B.A. Owiredu (SMS-KNUST and KATH) Dr.Christian Obirikorang (SMS-KNUST) Ametepe Samuel (SMS-KNUST).

Background (Please explain simply and briefly what the study is about):

Cardiovascular (heart) disease has become the number-one cause of death in the developing world. 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. The overall burden continues to grow in both developed and developing countries. 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. Diabetes prevalence studies in southern Ghana have recorded a steady increase. Diabetes screening conducted by the Ghana Diabetes Association in the early 1990s suggested 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 prediabetes was recorded in a community in Accra and lately in 2008, WHO also estimated that the prevalence of diabetics in the country stands at 8.8% among others **(FHI 360, 2012)** Inflammation has mostly been associated with diabetes and risks of developing heart disease(s). Measuring inflammation and vascular oxidative stress may provide clinicians with additional information regarding a patient's risk of CVD (cardiovascular disease).

Purpose(s) of research:

Therefore the study seeks to evaluate oxidative stress and inflammatory markers in type 2 diabetics and hypertensive at Shai-Osudoku District Hospital, Dodowa, Greater Accra, Ghana.

Procedure of the research, what shall be required of each participant and approximate total number of participants that would be involved in the research:

Questionnaires will be used to select participants into this study. Each selected participant will be required to fast overnight after which blood samples will be collected between 8:00am-10:00am. These samples will be analysed in the laboratory. Other physical measurements such as body weight, height etc. will also be performed on all qualified applicants. In total we expect to recruit 250 participants into this study at Shai-Osudoku District Hospital, Dodowa.

Risk(s)

Blood draw may cause some pain and/or minor bleeding to you but these are associated with all procedures that may require withdrawal of blood specimen for screening and/or diagnosis. That notwithstanding, every care will be taken by the researcher to minimise the discomfort that may be associated with this procedure and in the unlikely event that some complications develop in the process, the participant will be treated.

Benefit(s):
The benefits are two-fold. Not only are you going to have your risk of developing heart diseases and diabetes assessed through physical examination and laboratory testing but you will be thoroughly educated on the risks of developing these diseases and their prevention. Participants at high risk will be notified and referred for proper additional examinations and treatment.

Confidentiality:
All information collected in this study will be given code numbers. No name will be recorded. Data collected cannot be linked to you in anyway. No name or identifier will be used in any publication or reports from this study. However, as part of our responsibility to conduct this research properly, we may allow officials from ethics committees to have access to your records.

Voluntariness:
Taking part in this study should be out of your own free will. You are not under obligation to participate. Research is entirely voluntary.

Alternatives to participation:
If you choose not to participate, this will not affect your treatment in this hospital/institution in any way.

Withdrawal from the research:
You may choose to withdraw from the research at anytime without having to explain yourself. You may also choose not to answer any question you find uncomfortable or private.

Consequence of Withdrawal:
There will be no consequence, loss of benefit or care to you if you choose to withdraw from the study. Please note however, that some of the information that may have been obtained from you without identifiers (name etc), before you chose to withdraw, may have been modified or used in analysis reports and publications. These cannot be removed anymore. We do promise to make good faith effort to comply with your wishes as much as practicable.

Costs/Compensation:
For your time/inconvenience/transport to the hospital, we will compensate you with GH¢2.00 to show our appreciation for your participation.

Contacts:
If you have any question concerning this study, please do not hesitate to contact Ametepe Samuel (Name of Researcher) on (0244969598).

Further, if you have any concern about the conduct of this study, your welfare or your rights as a research participant, you may contact:

The Office of the Chairman
Committee on Human Research and Publication Ethics
Kumasi
Tel: 03220 63248 or 020 5453785

CONSENT FORM

Statement of person obtaining informed consent:

I have fully explained this research to _____ and have given sufficient information about the study, including that on procedures, risks and benefits, to enable the prospective participant make an informed decision to or not to participate.

DATE: _____ NAME: _____

Statement of person giving consent:
I have read the information on this study/research or have had it translated into a language I understand. I have also talked it over with the interviewer to my satisfaction.

I understand that my participation is voluntary (not compulsory).

I know enough about the purpose, methods, risks and benefits of the research study to decide that I want to take part in it.

I understand that I may freely stop being part of this study at any time without having to explain myself.

I have received a copy of this information leaflet and consent form to keep for myself.

NAME:_____

DATE: _____ SIGNATURE/THUMB PRINT: _____

Statement of person witnessing consent (Process for Non-Literate Participants):

I ——————————————(Name of Witness) certify that information given to

_____(Name of Participant), in the local language, is a true reflection of what l have read from the study Participant Information Leaflet, attached.

WITNESS' SIGNATURE (maintain if participant is non-literate): _____

MOTHER'S SIGNATURE (maintain if participant is under 18 years): _____

MOTHER'S NAME: _____

FATHER'S SIGNATURE (maintain if participant is under 18 years): _____

FATHER'S NAME: _____

QUESTIONNAIRE / REPORT

REF. CODE.

A. PERSONAL DETAILS

Residence:…….
Age: …… Sex:….. Marital Status:……..
Body Weight (BW):…….. Height:……. Body Mass Index:……
Waist Circumference (WC):…… Hip Circumference:…. W/HR:….
Blood Pressure (BP): ……

B. EDUCATIONAL BACKGROUND AND OCCUPATION

Non Formal ☐ Basic ☐ Secondary ☐ Tertiary ☐ Post Graduate ☐
Nature of Work:……..
How many hours do you work?.....

C. PERSONAL HISTORY

Have you been diagnosed of hypertension? (Yes /No)
If yes, for how long?.....
Do you exercise? (Yes/No). If yes, how often?.....
Do you drink alcohol currently? (Yes/No)
If No, have you ever drunk alcohol? (Yes/No)
If yes , for how long?.....
Type of alcoholic beverage consumed:
……
How often do you drink? Social Drinker ☐
Heavy Drinker ☐ Moderate drinker ☐

Do you smoke currently? (Yes/No)
If yes, how long have you been smoking? .....
How many sticks per day? .....
If no, have you ever smoked? (Yes / No)
If yes, when did you stop? .....
Have you been diagnosed of diabetes? (Yes / No)
If yes, how long?.....
Have you been diagnosed of any inflammatory disease such as the following?
Rheumatoid Arthritis Asthma
Cardiovascular disease Hepatitis
Dermatitis/Skin Disease Kidney disease
Pelvic Inflammatory Disease Infection
Have you had any surgery recently? (Yes/No)
If yes, how long ago?.....
For female participants only;
Are you currently pregnant? (Yes/No)
If yes, for how long?.....

D. TREATMENT / MEDICATION

Are you on medication for the following conditions?

Hypertension	Cancer
Diabetes	Rheumatoid Arthritis
Cardiovascular disease	Hepatitis
Pelvic Inflammatory Disease	Kidney disease
Infection	Dermatitis

E.NUTRITION / FOOD FREQUENCY

Salt intake: Moderate ☐ High ☐ Very High ☐

Sugar intake: Moderate ☐ High ☐ Very High ☐

Dietary (Animal) Fat intake: Moderate ☐ High ☐ Very High ☐

F.FAMILY HISTORY

Diabetes
Hypertension
Cardiovascular disease
Kidney disease
Smoking
Alcoholism
Obesity