STUDIES ON THE LEVELS OF LIPID PEROXIDATION AND TOTAL ANTIOXIDANT STATUS OF DIABETIC AND HYPERTENSIVE SUBJECTS: A CASE STUDY OF ADAMAWA STATE

BY

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DECLARATION

I hereby declare that this project report was written by me and it is a record of my own research work. It has not been presented before in any previous application for a higher degree. All references cited have been duly acknowledged

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Kinjir, Hauwa Date

DEDICATION

I dedicate this project to the Almighty God for seeing me through the program.

APPROVAL

This project report entitled "Studies on the Levels of Lipid Peroxidation and total Antioxidant status of Diabetic and Hypertensive Subjects: A case study of Adamawa State" meets the regulation governing the award of Masters of Technology of Modibbo Adama University of Technology, Yola and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

In this study, biochemical investigations were carried out in the blood samples of 200 subjects. 50 diabetes patients, 50 hypertensive patients, 50 hypertensive-diabetes patients and 50 control subjects were enrolled in this study. The levels of glucose, lipid peroxide, total cholesterol and LDL were found to significantly increase ($p<0.05$) in diabetes and hypertension-diabetes patients when compared to the values of control subjects. While the levels of total antioxidant status and HDL were found to significantly decrease ($p<0.05$) in both diabetes and hypertension-diabetes subjects when compared to values of control subjects. The levels of lipid peroxide, total cholesterol and LDL were also found to be significantly increase ($p<0.05$) in hypertensive patients when compared to values of control subject. However total antioxidant and HDL significant decrease ($p<0.05$) when compared to values of control subjects. Significant increase ($p<0.05$) in glucose, lipid peroxide, total cholesterol and LDL of 36-55 year old patients were observed when compared to 20-35 year old patients. Also there was significant decrease ($p<0.05$ in total antioxidant and HDL of 36-55 year old patients when compared to 20-35 years old. Negative correlation between serum total antioxidant and HDL were observed in serum glucose level of diabetic patients. Positive correlation between lipid peroxide, total cholesterol, triglyceride and LDL were found among diabetic patients. In hypertensive-diabetic patients, there was negative correlation between serum total antioxidant, HDL and serum glucose. Positive correlation between lipid peroxidation, total cholesterol, LDL and serum glucose were seen among hypertensive-diabetes patients. This data suggests the involvement of oxidative stress in the pathophysiology of diabetes, revealing that the increased lipid peroxidation has a close relationship with high glucose levels. The results show a correlation between total antioxidant, lipid peroxidation and lipid profile in diabetes, hypertensive and diabetes-hypertensive patients. It is concluded that the aging process is accompanied by elevated oxidative stress levels and could be a factor in instigating both disease conditions. On the other hand the disease conditions may appear to accelerate the age dependent increased oxidative stress.

LIST OF ABBREVIATIONS

Abs	Absorbance
ADH	Antidiuretic hormone
AGE	Advance glycalated end
APO B	Apoliprotien blood
AVP	Arginine Vasopressin
BP	Blood Pressure
BXCO	Biovex company
Cat No	Catalogue number
CD	Conjugated diene
Concn	Concentration
COX_2	Cyclooxygenase
DASH	Dietary approaches stop hypertension
DI	Diabetes insipidus
DM	Diabetes mellitus
DNA	Deoxyribonucleic acid
DPPIV	Dipeptidyl peptidase IV
GDM	Gestational Diabetes mellitus
GIP	Gastric inhibitory polypeptide
GLIP	Glucagon-like inhibitory peptide
GLP-1	Glucagon-like peptide-1/Action
GLU	Glucose

HbALC	Glycated haemoglobin
HCLO	Hypochlorous acid
HDL	High density lipoprotein
HLA	Human leucocytes antigen
HOD	Head of department
HTN	Hypertension
IDDM	Insulin dependent diabetes mellitus
IRSI	Insulin receptor substrate
LADA	Latent autoimmune diabetes of adults
LDL	Low density lipoprotien
MDA	Malondialdehyde
MMDM	Malnutrition modulated diabetes mellitus
mmHg	millimeter mercury
MRDM	Malnutrition-related diabetes mellitus
NADPH	Nitrogen adesine dihydrogen phosphate
Na EDTA	Sodium ethylene dicitrate acid
NHES	National Health examination survey
NIDDM	Non-insulin dependent diabetes mellitus
NO	Nitric oxide
NS	Nitrogen species
OD	Optical density
PUFAS	Polyunsaturated fatty acids
Q	Quinine

NS	Nitrogen species
R	Reagents
RAAS	Renin-agiotensin-aldosterone system
RNS	Reactive oxygen species
ROS	Reactive oxygen species
SHR	Hypertensive rat
SNS	Sympathetic nervous system
SOD	Superoxide dismutase
STD	Standard
TAS	Total antioxidant status
TBA	Thiobarbituric acid
TCA	Trichloroacetic acid
WHO	World health organization
WKY	Wistar-kyoto

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CHAPTER ONE

INTRODUCTION

Hypertension (HTN) or high blood pressure is a cardiac chronic medical condition in which the systemic arterial blood pressure is elevated. It is the opposite of hypotension. Hypertension is classified as either primary (essential) or secondary. About 90 – 95% of cases are termed "primary hypertension", which refers to high blood pressure for which no medical cause can be found (Carretero and Oparil, 2000). The remaining 5–10% of cases (secondary hypertension) is caused by other conditions that affect the kidneys, arteries, heart, or endocrine system (Mayo foundation for medical education and medical research, 2008).

Persistent hypertension is one of the risk factors for stroke, myocardial infarction, heart failure and arterial aneurysm and is a leading cause of chronic kidney failure (Pierdomenico, 2009). Moderate elevation of arterial blood pressure leads to shortened life expectancy. Dietary and lifestyle changes can improve blood pressure control and decrease the risk of associated health complications, although drug treatment may prove necessary in patients for whom lifestyle changes prove ineffective or insufficient (Pierdomenico, 2009).

Diabetes mellitus, often simply referred to as diabetes is a group of metabolic diseases in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced. This high blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger). There are three main types of diabetes (Fowler, 2007).

Type 1 diabetes results from the body's failure to produce insulin, and presently requires the person to inject insulin (Also referred to as *insulin-dependent* diabetes mellitus, *IDDM* and *juvenile* diabetes (Expert Committee on Diagnosis and Classification of Diabetes Mellitus, 2002).

Type 2 diabetics results from insulin resistance, a condition in which cells fail to use insulin properly, sometimes combined with an absolute insulin deficiency. (Formerly

referred to as *non-insulin-dependent* diabetes mellitus, *NIDDM* and *adult-onset* diabetes) (America diabetes association, 2007).

Gestational diabetes: is when pregnant women, who have never had diabetes before but have high blood glucose level during pregnancy. It may precede development of type 2 diabetes mellitus (DM) (Kim *et al*., 2002).

Other forms of diabetes mellitus include congenital diabetes, which is due to genetic defects of insulin secretion, cystic fibrosis-related diabetes, steroid diabetes induced by high doses of glucocorticoids, and several forms of monogenic diabetes (American Diabetes Association Diagnosis and Classification, 2007).

All forms of diabetes have been treatable since insulin became available in 1921, and type 2 diabetes may be controlled with medications. Both type 1 and 2 are chronic conditions that usually cannot be cured. Pancreas transplants have been tried with limited success in type 1diabetes mellitus (DM); gastric bypass surgery has been successful in many with morbid obesity and type 2 diabetes mellitus Gestational diabetes usually resolves after delivery. Diabetes without proper treatments can cause many complications. Acute complications include hyperglycemia, diabetic ketoacidosis, or non ketotic hyperosmolar coma. Serious long-term complications include cardiovascular disease, chronic renal failure, and retinal damage. Adequate treatment of diabetes is thus important, as well as blood pressure control and lifestyle factors such as smoking cessation and maintaining a healthy body weight (Kim *et al.*, 2002).

In 2000 at least 171 million people worldwide suffer from diabetes, or 2.8% of the population (Wild, 2004). Type 2 (DM) is by far the most common, affecting 90 to 95% of the U.S. diabetes population (Knowler *et al*., 2002).

The relationship between hypertension, oxidative stress and antioxidants is complex and inadequately understood. Oxidative stress may play a role in the pathophysiology of hypertension, as has been shown in animal models and differing mechanisms have been suggested for this (Griedling and Fitzgerald, 2003). The evidence for this in humans is however not definitive (Oparil *et al.*, 2003). Hypertension, on the other hand, may lead to tissue damage through lipid peroxidation and other oxidative mechanisms (Kumar and Das, 1993). Research suggests that *in vivo* oxidation of low-

density lipoproteins, primarily by oxygen-free radicals, may increase atherogenesis (Beckman and Ames, 1998; Jessup *et al.*, 1993; Maxwell, 1993). Hypertension associated with long-term infusion of angiotensin II is linked to the stimulation of oxidant production, leading to reduced bioactivity of nitric oxide (NO) and the activation of NADPH oxidase (Oparil *et al.*, 2003).

The relationship of hypertension and diabetes with antioxidants and oxidative stress is therefore of much interest. In a systematic review of antioxidants and prevention of cardiovascular disease, there was no evidence of an association between blood pressure (BP) and intakes of either carotene or Vitamin E (Asplund, 2002). However, findings on associations between Vitamin C levels and hypertension have been more mixed, with the general suggestion that low intake of dietary Vitamin C may be related to an increased risk of hypertension. While Vitamin C administration was found to be associated with lower BP in a randomised control trial, a further exploration of the relationship between long-term Vitamin C supplementation and BP did not support this finding (Oparil *et al.*, 2003). Two reviews on this topic have concluded that such an association between intake of Vitamin C and hypertension exists, but could be the result of other confounding factors (Asplund, 2002; Ness, 1997).

Lipid peroxidation and derived oxidized products are being intensively investigated, because of their potential to cause injury and their pathogenetic role in several clinically significant diseases. The view that an excess of lipid peroxidation products is present and relevant in the pathogenesis of human essential hypertension or in hypertension-induced damage and diabetes has still not received definitive support. Also some large studies have reported on the associations between antioxidants and cardiovascular disease, myocardial infarction and mortality (Klipstein *et al.*, 1998; Yochum *et al.*, 2000). Fewer studies have focused on the relationship between antioxidant measures and hypertension, hence the need to evaluate the antioxidant status and lipid peroxidation levels in hypertensive and diabetic patients (Oparil, *et al.*, 2003).

Both diabetes mellitus, hypertension and oxidative stress are conditions more frequently observed in older persons, (Ames *et al.*, 2003). Furthermore, oxidative stress has been proposed as a mediator or a causative mechanism of the aging process itself

(Lewington *et al.,* 2003; Ames *et al*., 2003). Diabetes and hypertension are characterized by an acceleration of vascular alterations and are major risk factors for cardiovascular disease (Yach *et al*., 2004). The most powerful risk factor for cardiovascular death and hypertension in large populations is age itself (Lewington *et al*., 2003). Ligia *et al*., (2010) hypothesized that aging shares common characteristic with type 2 diabetes and hypertension, suggesting that diabetes and hypertension may cause an acceleration of the nature of age dependent increase in oxidative stress and oxidative damage (Zimmet *et al.*, 2009).

1.1 Justification of the study

The relationship between diabetics, hypertension, oxidative stress and antioxidants is complex and inadequately understood. Oxidative stress may play a role in the pathophysiology of hypertension, as has been shown in animal models and differing mechanisms have been suggested. The evidence for this in humans is however not definitive

1.2 Aim and Objectives

The aim of the research is to study different biochemical parameters in the serum of diabetics, hypertensive and combined diabetics plus hypertensive condition among different age groups.

The specific objectives are:

i. To determine the levels of lipid peroxidation, antioxidant and lipid profile of diabetic, hypertensive and diabetic-hypertensive patients.

ii. To determine whether there is/are association(s) between glucose and total antioxidants, lipid peroxidation and lipid profiles in diabetics, hypertensive and diabetic-hypertensive conditions.

CHAPTER TWO
LITERATURE REVIEW

2.1 Hypertension

Hypertension (HTN) is a cardiac chronic medical condition in which the systemic arterial blood pressure is elevated. It is the opposite of hypotension. Blood pressure is usually classified based on the systolic and diastolic blood pressures. Systolic blood pressure is the blood pressure in vessels during a heart beat. Diastolic blood pressure is the pressure between heartbeats. A systolic or the diastolic blood pressure measurement higher than the accepted normal values for the age of the *individual* is classified as prehypertension or hypertension (Gibson, 2009).

Hypertension has several sub-classifications including, hypertension stage I, hypertension stage II, and isolated systolic hypertension. Isolated systolic hypertension refers to elevated systolic pressure with normal diastolic pressure and is common in the elderly. These classifications are made after averaging a patient's resting blood pressure readings taken on two or more office visits. Individuals older than 50 years are classified as having hypertension if their blood pressure is consistently at least 140 mmHg systolic or 90 mmHg diastolic. Patients with blood pressures higher than 130/80 mmHg with concomitant presence of diabetes mellitus or kidney disease require further treatment. Hypertension is also classified as resistant if medications do not reduce blood pressure to normal levels (Brixi, *et al*., 2011).

Exercise hypertension is an excessively high elevation in blood pressure during exercise. The range considered normal for systolic values during exercise is between 200 and 230 mmHg. Exercise hypertension may indicate that an individual is at risk for developing hypertension at rest (Gibson, 2009).

2.1.1 Signs and symptoms of hypertension

Mild to moderate essential hypertension is usually asymptomatic (Pitts and Adams, 1998). Accelerated hypertension is associated with headache, drowsiness, confusion, vision disorders, nausea, and vomiting symptoms which are collectively referred to as hypertensive encephalopathy. Hypertensive encephalopathy is caused by

severe small blood vessel congestion and brain swelling, which is reversible if blood pressure is low (Papadakis and Mcphee, 2008).

Some signs and symptoms are especially important in newborns and infants such as failure to thrive, seizures, irritability, lack of energy, and difficulty in breathing. In children, hypertension can cause headache, fatigue, blurred vision, nosebleeds, and facial paralysis (Rodriguez and Ettinger, 2010). Even with the above clinical symptoms, the true incidence of pediatric hypertension is not known. In adults, hypertension has been defined due to the adverse effects caused by hypertension. However, in children, similar studies have not been performed thoroughly to link any adverse effects with the increase in blood pressure. Therefore, the prevalence of pediatric hypertension remains unknown due to the lack of scientific knowledge.

Some additional signs and symptoms suggest that hypertension is caused by disorders in hormone regulation. Hypertension combined with obesity distributed on the trunk of the body, accumulated fat on the back of the neck ('buffalo hump'), wide purple marks on the abdomen (abdominal striate), or the recent onset of diabetes suggests that an individual has a hormone disorder known as Cushing's syndrome. Hypertension caused by other hormone disorders such as hyperthyroidism, hypothyroidism, or growth hormone excess will be accompanied by additional symptoms specific to these disorders. For example, hyperthyrodism can cause weight loss, tremors, heart rate abnormalities, reddening of the palms, and increased sweating (Brixi *et al*., 2011). Signs and symptoms associated with growth hormone excess include coarsening of facial features, protrusion of the lower jaw, enlargement of the tongue, excessive hair growth, darkening of the skin color, and excessive sweating (James *et al*., 2005). Other hormone disorders like hyperaldosteronism may cause less specific symptoms such as numbness, excessive urination, excessive sweating, electrolyte imbalances and dehydration, and elevated blood alkalinity and also cause of mental pressure (Chrouse and Lafferty, 2009).

Hypertension in pregnant women is one symptom of pre-eclampsia. Pre-eclampsia can progress to a life-threatening condition called eclampsia, which is the development of protein in the urine, generalized swelling, and severe seizures. Other symptoms indicating that brain function is becoming impaired may precede these

seizures such as nausea, vomiting, headaches, and vision loss (Gibson, 2009). In addition, the systemic vascular resistance and blood pressure decrease during pregnancy. The body must compensate by increasing cardiac output and blood volume to provide sufficient circulation in the utero-placental arterial bed (Hana *et al.*, 2011).

2.1.2 Glucose metabolism and regulation

Diabetes as a metabolic disease has evolved significantly since the discovery of insulin in the 1920s. Insulin was identified as a potent hormonal regulator of both glucose appearance and disappearance in the circulation. Subsequently, diabetes was viewed as a mono-hormonal disorder characterized by absolute or relative insulin deficiency. Since its discovery, insulin has been the only available pharmacological treatment for patients with type 1 diabetes and a mainstay of therapy for patients with insulin-deficient type 2 diabetes *(American Diabetes Association: 2004).*The recent discovery of additional hormones with glucoregulatory actions has expanded our understanding of how a variety of different hormones contribute to glucose homeostasis. In the 1950s, glucagon was characterized as a major stimulus of hepatic glucose production. This discovery led to a better understanding of the interplay between insulin and glucagon, thus leading to a bi-hormonal definition of diabetes. Subsequently, the discovery of a second Beta-cell (β-cell) hormone, amylin, was first reported in 1987. Amylin was determined to have a role that complemented that of insulin, and, like insulin, was found to be deficient in people with diabetes. This more recent development led to a view of glucose homeostasis involving multiple pancreatic hormones (Moore and Cooper, 1999).

2.1.3 Normal physiology

Plasma glucose concentration is a function of the rate of glucose entering the circulation (glucose appearance) balanced by the rate of glucose removal from the circulation (glucose disappearance). Circulating glucose is derived from three sources: intestinal absorption during the fed state, glycogenolysis, and gluconeogenesis. The major determinant of how quickly glucose appears in the circulation during the fed state is the rate of gastric emptying. Other sources of circulating glucose are derived chiefly

from hepatic processes: glycogenolysis, the breakdown of glycogen, the polymerized storage form of glucose; and gluconeogenesis, the formation of glucose primarily from lactate and amino acids during the fasting state.

Glycogenolysis and gluconeogenesis are partly under the control of glucagon, a hormone produced in the α-cells of the pancreas. During the first 8–12 hours of fasting, glycogenolysis is the primary mechanism by which glucose is made available. Glucagon facilitates this process and thus promotes glucose appearance in the circulation. Over longer periods of fasting, glucose, produced by gluconeogenesis, is released from the liver.

Glucoregulatory hormones include insulin, glucagon, amylin, GLP-1, glucose-dependent insulinotropic peptide (GIP), epinephrine, cortisol, and growth hormone. Of these, insulin and amylin are derived from the β-cells, glucagon from the α-cells of the pancreas, and GLP-1 and GIP from the L-cells of the intestine.

The glucoregulatory hormones of the body are designed to maintain circulating glucose concentrations in a relatively narrow range. In the fasting state, glucose leaves the circulation at a constant rate. To keep pace with glucose disappearance, endogenous glucose production is necessary. For all practical purposes, the sole source of endogenous glucose production is the liver. Renal gluconeogenesis contributes substantially to the systemic glucose pool only during periods of extreme starvation. Although most tissues have the ability to hydrolyze glycogen, only the liver and kidneys contain glucose-6-phosphatase, the enzyme necessary for the release of glucose into the circulation. In the bi-hormonal model of glucose homeostasis, insulin is the key regulatory hormone of glucose disappearance, and glucagon is a major regulator of glucose appearance. After reaching a post-meal peak, blood glucose slowly decreases during the next several hours, eventually returning to fasting levels. In the immediate post-feeding state, glucose removal into skeletal muscle and adipose tissue is driven mainly by insulin. At the same time, endogenous glucose production is suppressed by (1) the direct action of insulin, delivered via the portal vein, on the liver, and (2) the paracrine effect or direct communication within the pancreas between the α- and β-cells, which results in glucagon suppression (Wallum *et al.,* 1992, Gerich 1993 and Lefebvre 1995).

2.1.4 Beta-cell hormones

i. Insulin

Until recently, insulin was the only pancreatic β-cell hormone known to lower blood glucose concentrations. Insulin, a small protein composed of two polypeptide chains containing 51 amino acids, is a key anabolic hormone that is secreted in response to increased blood glucose and amino acids following ingestion of a meal. Like many hormones, insulin exerts its actions through binding to specific receptors present on many cells of the body, including fat, liver, and muscle cells. The primary action of insulin is to stimulate glucose disappearance.

Insulin helps control postprandial glucose in three ways. Initially, insulin signals the cells of insulin-sensitive peripheral tissues, primarily skeletal muscle, to increase their uptake of glucose (Gerich, 1993). Secondly, insulin acts on the liver to promote glycogenesis. Finally, insulin simultaneously inhibits glucagon secretion from pancreatic α-cells, thus signalling the liver to stop producing glucose via glycogenolysis and gluconeogenesis.

ii Effects of primary glucoregulatory hormones

All of these actions reduce blood glucose. Other actions of insulin include the stimulation of fat synthesis, promotion of triglyceride storage in fat cells, promotion of protein synthesis in the liver and muscle, and proliferation of cell growth (Cryer, 1992).
Insulin action is carefully regulated in response to circulating glucose concentrations. Insulin is not secreted if the blood glucose concentration is ≤ 3.3 mMol/L, but is secreted in increasing amounts as glucose concentrations increase beyond this threshold (Gerich, 1993). Postprandially, the secretion of insulin occurs in two phases: an initial rapid release of preformed insulin, followed by increased insulin synthesis and release in response to blood glucose. Long-term release of insulin occurs if glucose concentrations remain high (Cryer, 1992).

While glucose is the most potent stimulus of insulin, other factors stimulate insulin secretion. These additional stimuli include increased plasma concentrations of

some amino acids, especially arginine, leucine, and lysine; GLP-1 and GIP released from the gut following a meal; and parasympathetic stimulation via the vagus nerve.

iii. Amylin

Isolated from pancreatic amyloid deposits in the islets of Langerhans, amylin was first reported in the literature in 1987. Amylin, a 37–amino acid peptide, is a neuroendocrine hormone co-expressed and co-secreted with insulin by pancreatic β-cells in response to nutrient stimuli (Koda *et al.,* 1992). When secreted by the pancreas, the insulin-to-amylin molar ratio in the portal circulation is approximately 50:1 because of hepatic extraction of insulin this ratio falls to approximately 20:1 in the peripheral circulation (Weyer *et al.,* 2001)

Studies in humans have demonstrated that the secretory and plasma concentration profiles of insulin and amylin are similar with low fasting concentrations and increases in response to nutrient intake (Fein *et al.,* 1984). In healthy adults, fasting plasma amylin concentrations range from 4 to 8 pmol/l rising as high as 25 pmol/l postprandially. In subjects with diabetes, amylin is deficient in type 1 and impaired in type 2 diabetes (Kruger *et al.,* 1999).

Preclinical findings indicate that amylin works with insulin to help coordinate the rate of glucose appearance and disappearance in the circulation, thereby preventing an abnormal rise in glucose concentrations (Pehling *et al.,* 1984).

Amylin complements the effects of insulin on circulating glucose concentrations via two main mechanisms. Amylin suppresses post-prandial glucagon secretion (Gedulin *et al.,* 1997), thereby decreasing glucagon-stimulated hepatic glucose output following nutrient ingestion. This suppression of post-prandial glucagon secretion is postulated to be centrally mediated via efferent vagal signals. Importantly, amylin does not suppress glucagon secretion during insulin-induced hypoglycemia (Samson *et al.,* 2000). Amylin also slows the rate of gastric emptying and, thus, the rate at which nutrients are delivered from the stomach to the small intestine for absorption (Samson *et al.,* 2000). In addition to its effects on glucagon secretion and the rate of gastric emptying, amylin dose-dependently reduces food intake and body weight in animal models. Amylin exerts its actions primarily through the central nervous system. Animal studies have identified

specific calcitonin-like receptor sites for amylin in regions of the brain, predominantly in the area postrema. The area postrema is a part of the dorsal vagal complex of the brain stem. A notable feature of the area postrema is that it lacks a blood-brain barrier, allowing exposure to rapid changes in plasma glucose concentrations as well as circulating peptides, including amylin (Beeley and Prickett, 1996; Wimalawansa, 1997).

In summary, amylin works to regulate the rate of glucose appearance from both endogenous (liver-derived) and exogenous (meal-derived) sources, and insulin regulates the rate of glucose disappearance (Buse *et al.*, 2002).

2.1.5 Alpha-cell hormone: Glucagon

Glucagon is a key catabolic hormone consisting of 29 amino acids. It is secreted from pancreatic alpha cell (α-cells). Described by Roger Unger in the 1950s, glucagon was characterized as opposing the effects of insulin (Unger, 1971). Glucagon plays a major role in sustaining plasma glucose during fasting conditions by stimulating hepatic glucose production.

Unger was the first to describe the diabetic state as a “bi-hormonal” disease characterized by insulin deficiency and glucagon excess. He further speculated that a therapy targeting the correction of glucagon excess would offer an important advancement in the treatment of diabetes (Unger, 1971).

Hepatic glucose production, which is primarily regulated by glucagon, maintains basal blood glucose concentrations within a normal range during the fasting state. When plasma glucose falls below the normal range, glucagon secretion increases, resulting in hepatic glucose production and return of plasma glucose to the normal range (Gerich, 1993).This endogenous source of glucose is not needed during and immediately following a meal and glucagon secretion is suppressed. When coupled with insulin's direct effect on the liver, glucagon suppression results in a near-total suppression of hepatic glucose output.

In the diabetic state, there is inadequate suppression of postprandial glucagon secretion (hyperglucagonemia) (Dinneen *et al.*, 1995). Resulting in elevated hepatic glucose production, importantly, exogenously administered insulin is unable both to

restore normal postprandial insulin concentrations in the portal vein and to suppress glucagon secretion through a paracrine effect. This results in an abnormally high glucagon-to-insulin ratio that favors the release of hepatic glucose (Baron *et al.*, 1998). These limits of exogenously administered insulin therapy are well documented in individuals with type 1 and type 2 diabetes are considered to be important contributors to the postprandial hyperglycemic state characteristic of diabetes.

2.1.6 Incretin hormones glucagon-like peptide-1 and gastric inhibitory polypeptide (GLP-1 and GIP)

The intricacies of glucose homeostasis become clearer when considering the role of gut peptides. By the late 1960s (Perley and Kipnis, 1967) and others demonstrated that ingested food caused a more potent release of insulin than glucose infused intravenously. This effect, termed the "incretin effect," suggested that signals from the gut are important in the hormonal regulation of glucose disappearance. Additionally, these hormonal signals from the proximal gut seemed to help regulate gastric emptying and gut motility. Several incretin hormones have been characterized, and the dominant ones for glucose homeostasis are GIP and GLP-1 GIP stimulates insulin secretion and regulates fat metabolism, but does not inhibit glucagon secretion or gastric emptying (Yip and Wolfe, 2000). GIP levels are normal or slightly elevated in people with type 2 diabetes (Visboll *et al.*, 2001). While GIP is a more potent incretin hormone, GLP-1 is secreted in greater concentrations and is more physiologically relevant in humans (Nauck *et al.*, 2002).

GLP-1 also stimulates glucose-dependent insulin secretion but is significantly reduced postprandially in people with type 2 diabetes or impaired glucose tolerance (Lugari *et al.*, 2002) GLP-1 stimulates insulin secretion when plasma glucose concentrations are high but not when plasma glucose concentrations approach or fall below the normal range. Derived from the proglucagon molecule in the intestine, GLP-1 is synthesized and secreted by the L-cells found mainly in the ileum and colon. Circulating GLP-1 concentrations are low in the fasting state. However, both GIP and GLP-1 are effectively stimulated by ingestion of a mixed meal or meals enriched with

fats and carbohydrates (Herrmann *et al.,* 1995). In contrast to GIP, GLP-1 inhibits glucagon secretion and slows gastric emptying (Drucker and Minireviel, 2001).

GLP-1 has many glucoregulatory effects. In the pancreas, GLP-1 stimulates insulin secretion in a glucose-dependent manner while inhibiting glucagon secretion. (Perfetti and Merkel, 2002). Animal studies have demonstrated that the action of GLP-1 occurs directly through activation of GLP-1 receptors on the pancreatic β-cells and indirectly through sensory nerves. (Rachman *et al.,* 1996). GLP-1 has a plasma half-life of about 2 minutes, and its disappearance is regulated primarily by the enzyme dipeptidyl peptidase-IV (DPP-IV), which rapidly cleaves and inactivates GLP-1.

Infusion of GLP-1 lowers postprandial glucose as well as overnight fasting blood glucose concentrations. The postprandial effect of GLP-1 is partly due to inhibition of glucagon secretion. Yet while GLP-1 inhibits glucagon secretion in the fed state, it does help regulate gastric emptying and gastric acid secretion (Drucker and Minireviel, 2001), perhaps by signalling GLP-1 receptors in the brain and thereby stimulating efferent tracts of the vagus nerve (Nauck *et al.,* 2002). As gastric emptying slows, the postprandial glucose excursion is reduced. Administration of GLP-1 has been associated with the regulation of feeding behavior and body weight. In addition, there have been reported observations of GLP-1 improving insulin sensitivity and enhancing glucose disposal (Zander *et al.,* 2002).Of significant and increasing interest is the role GLP-1 may have in preservation of β-cell function and β-cell proliferation. In animal studies, GLP-1 has been shown to enhance functional β-cell mass (Drucker, 2003).

2.2 Diabetes pathophysiology

The understanding of the pathophysiology of diabetes is evolving. Type1 diabetes has been characterized as an autoimmune-mediated destruction of pancreatic β-cells (Atkinson and Maclaren, 1994). The resulting deficiency in insulin also means a deficiency in the other cosecreted and colocated β-cell hormone, amylin (Kruger *et al.*, 1999). As a result, postprandial glucose concentrations rise due to lack of insulin-stimulated glucose disappearance, poorly regulated hepatic glucose production and increased or abnormal gastric emptying following a meal (Kruger *et al.,* 1999).

Early in the course of type2 diabetes, postprandial β-cell action becomes abnormal, as evidenced by the loss of immediate insulin response to a meal (Kahn 2000). Peripheral insulin resistance coupled with progressive β-cell failure and decreased availability of insulin, amylin, and GLP-1 (Toft *et al.,* 2001). Contribute to the clinical picture of hyperglycemia in diabetes.

Abnormal gastric emptying is common to both type 1 and type2 diabetes. The rate of gastric emptying is a key determinant of postprandial glucose concentrations. If gastric emptying is accelerated, then the presentation of meal-derived glucose to the circulation is poorly timed with insulin delivery. In individuals with diabetes, the absent or delayed secretion of insulin further exacerbates postprandial hyperglycemia. Both amylin and GLP-1 regulate gastric emptying by slowing the delivery of nutrients from the stomach to the small intestine (Stephen *et al.,* 200*4*).

2.2.1 Regulation of glucagon action

Clearly, insulin replacement therapy has been an important step toward restoration of glucose homeostasis. But it is only part of the ultimate solution. The vital relationship between insulin and glucagon has suggested additional areas for treatment. With inadequate concentrations of insulin and elevated concentrations of glucagon in the portal vein, glucagon's actions are excessive, contributing to an endogenous and unnecessary supply of glucose in the fed state. To date, no pharmacological means of regulating glucagon exist and the need to decrease postprandial glucagon secretion remains a clinical target for future therapies (Stephen *et al*., 2004).

2.2.2 Amylin actions

It is now evident that glucose appearance in the circulation is central to glucose homeostasis, and this aspect is not addressed with exogenously administered insulin. Amylin works with insulin and suppresses glucagon secretion. It also helps regulate gastric emptying, which in turn influences the rate of glucose appearance in the circulation. A synthetic analog of human amylin that binds to the amylin receptor, an amylinomimetic agent, is in development.

2.2.3 Glucagon-like peptide-1 actions (GLP-1)

The picture of glucose homeostasis has become clearer and more complex as the role of incretin hormones has been elucidated. Incretin hormones play a role in helping regulate glucose appearance and in enhancing insulin secretion. Secretion of GIP and GLP-1 is stimulated by ingestion of food, but GLP-1 is the more physiologically relevant hormone (Holst 1994).

However, replacing GLP-1 in its natural state poses biological challenges. In clinical trials, continuous subcutaneous or intravenous infusion was superior to single or repeated injections of GLP-1 because of the rapid degradation of GLP-1 by DPP-IV.

To circumvent this intensive and expensive mode of treatment, clinical development of compounds that elicit similar glucoregulatory effects to those of GLP-1 are being investigated. These compounds, termed incretin mimetics, have a longer duration of action than native GLP-1. In addition to incretin mimetics, research indicates that DPP-IV inhibitors may improve glucose control by increasing the action of native GLP-1. These new classes of investigational compounds have the potential to enhance insulin secretion and suppress prandial glucagon secretion in a glucose-dependent manner, regulate gastric emptying, and reduce food intake (Drucker, 2003).

2.3 Diabetes mellitus

The word diabetes is from the Greek *diabanein* which means to pass through, in reference to the excessive urine produced as a symptom of these diseases. The term *diabetes*, without qualification, usually refers to diabetes mellitus, which roughly translates to excessive sweet urine (known as "glycosuria"). Several rare conditions are also named diabetes. The most common of these is diabetes insipidus in which large amounts of urine are produced (polyuria), which is not sweet (insipidus meaning "without taste" in Latin) (Perspectives in Gestational Diabetes Mellitus, 2007).

The term "type 1 diabetes" has replaced several former terms, including childhood-onset diabetes, juvenile diabetes, and insulin-dependent diabetes mellitus (IDDM). Likewise, the term "type2 diabetes" has replaced several former terms,

including adult-onset diabetes, obesity-related diabetes, and non-insulin-dependent diabetes mellitus (NIDDM). Beyond these two types, there is no agreed-upon standard nomenclature. Various sources have defined "type 3 diabetes" as: gestational diabetes, (Trujillo *et al.*, 2008). Insulin-resistant type 1 diabetes (or "double diabetes"), type 2 diabetes which has progressed to require injected insulin, and latent autoimmune diabetes of adults (LADA or "type 1.5" diabetes (Zimmet *et al*., 2009).

2.3.1 Classification of diabetes

Most cases of diabetes mellitus fall into three broad categories: type 1, type 2, and gestational diabetes. A few other types are described (Cooke and Plotnick, 2008).

i. Type 1 diabetes

Type 1 diabetes mellitus is characterized by loss of the insulin-producing beta cells of the islets of Langerhans in the pancreas leading to insulin deficiency. This type of diabetes can be further classified as immune-mediated or idiopathic. The majority of type 1 diabetes is of the immune-mediated nature, where beta cell loss is a T-cell mediated autoimmune attack (Rother, 2007). There is no known preventive measure against type 1 diabetes, which causes approximately 10% of diabetes mellitus cases in North America and Europe. Most affected people are otherwise healthy and of a healthy weight when onset occurs. Sensitivity and responsiveness to insulin are usually normal, especially in the early stages. Type 1 diabetes can affect children or adults but was traditionally termed "juvenile diabetes" because it represents a majority of the diabetes cases in children (American Diabetes Association diagnosis and classification, 2007).

ii. Type 2 diabetes

Type 2 diabetes mellitus is characterized by insulin resistance which may be combined with relatively reduced insulin secretion. The defective responsiveness of body tissues to insulin is believed to involve the insulin receptor. However, the specific defects are not known. Diabetes mellitus due to a known defect are classified separately. Type 2 diabetes is the most common type (American Diabetes Association, 2004).

In the early stage of type 2 diabetes, the predominant abnormality is reduced insulin sensitivity. At this stage hyperglycemia can be reversed by a variety of measures and medications that improve insulin sensitivity or reduce glucose production by the liver (Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2002).

iii. Gestational diabetes

Gestational diabetes mellitus (GDM) resembles type 2 diabetes in several respects, involving a combination of relatively inadequate insulin secretion and responsiveness. It occurs in about 2%–5% of all pregnancies and may improve or disappear after delivery. Gestational diabetes is fully treatable but requires careful medical supervision throughout the pregnancy. About 20%–50% of affected women develop type 2 diabetes later in life. Even though it may be transient, untreated gestational diabetes can damage the health of the fetus or mother. Risks to the baby include macrosomia (high birth weight), congenital cardiac and central nervous system anomalies, and skeletal muscle malformations. Increased fetal insulin may inhibit fetal surfactant production and cause respiratory distress syndrome. Hyperbilirubinemia may result from red blood cell destruction. In severe cases, perinatal death may occur, most commonly as a result of poor placental perfusion due to vascular impairment. Labour induction may be indicated with decreased placental function. A cesarean section may be performed if there is marked fetal distress or an increased risk of injury associated with macrosomia, such as shoulder dystocia (Perspectives in Gestational Diabetes Mellitus, 2007). A 2008 study completed in the U.S. found that the number of American women entering pregnancy with preexisting diabetes is increasing. In fact the rate of diabetes in expectant mothers has more than doubled in the past 6 years. This is particularly problematic as diabetes raises the risk of complications during pregnancy, as well as increasing the potential that the children of diabetic mothers will also become diabetic in the future (Handelsman, 2009).

iv Diabetes insipidus (DI)

Diabetes insipidus is a condition characterized by excessive thirst and excretion of large amounts of severely diluted urine, with reduction of fluid intake having no effect on the concentration of the urine. There are several different types of DI, each with a different cause. The most common type in humans is central DI, caused by a deficiency of arginine vasopressin (AVP), also known as antidiuretic hormone (ADH). The second common type of DI is nephrogenic diabetes insipidus, which is caused by an insensitivity of the kidneys to ADH. It can also be an iatrogenic artifact of drug use. Although they have a common name, diabetes mellitus and diabetes insipidus are two entirely separate conditions with unrelated mechanisms. Both cause large amounts of urine to be produced (polyuria), and the term "diabetes" is derived from the Greek name for this symptom. However, diabetes insipidus is either a problem with the production of antidiuretic hormone (central diabetes insipidus) or kidney's response to antidiuretic hormone (nephrogenic diabetes insipidus), whereas diabetes mellitus causes polyuria via a process called osmotic diuresis, due to the high blood sugar leaking into the urine and taking excess water along with it. The incidence of diabetes insipidus in the general population is three in 100,000 (Saborio, *et al.*, 2000).

v. Other types of diabetes

Pre-diabetes indicates a condition that occurs when a person's blood glucose levels are higher than normal but not high enough for a diagnosis of type 2 diabetes. Many people destined to develop type 2 diabetes spend many years in a state of pre-diabetes which has been termed "America's largest healthcare epidemic. Latent autoimmune diabetes of adults (LADA) is a condition in which Type 1 diabetes develops in adults. Adults with LADA are frequently initially misdiagnosed as having Type 2 diabetes, based on age rather than etiology. Some cases of diabetes are caused by the body's tissue receptors not responding to insulin (even when insulin levels are normal, which is what separates it from type 2 diabetes); this form is very uncommon. Genetic mutations (autosomal or mitochondrial) can lead to defects in beta cell function. Abnormal insulin action may also have been genetically determined in some cases. Any disease that causes

extensive damage to the pancreas may lead to diabetes (for example, chronic pancreatitis and cystic fibrosis). Diseases associated with excessive secretion of insulin-antagonistic hormones can cause diabetes (which is typically resolved once the hormone excess is removed). Many drugs impair insulin secretion and some toxins damage pancreatic beta cells. The diagnostic entity, *malnutrition-related diabetes mellitus* (MRDM or MMDM, ICD-10 code E12), was deprecated by the World Health Organization when the current taxonomy was introduced in 1999 (WHO, 1999).

2.3.2 Signs and symptoms of diabetes

The classical symptoms of diabetes are polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger) (Cooke and Plotnick, 2008). Symptoms may develop rapidly (weeks or months) in type 1 diabetes while in type 2 diabetes they usually develop much more slowly and may be subtle or absent. Prolonged high blood glucose causes glucose absorption, which leads to changes in the shape of the lenses of the eyes, resulting in vision changes; sustained sensible glucose control usually returns the lens to its original shape. Blurred vision is a common complaint leading to a diabetes diagnosis; type 1 should always be suspected in cases of rapid vision change, whereas with type 2 changes are generally more gradual, but should still be suspected (NHES, 2011).

People (usually with type 1 diabetes) may also present with diabetic ketoacidosis, a state of metabolic dysregulation characterized by the smell of acetone; a rapid, deep breathing known as Kussmaul breathing; nausea; vomiting and abdominal pain; and an altered states of consciousness. A rarer but equally severe possibility is hyperosmolar non ketotic state, which is more common in type 2 diabetes and is mainly the result of dehydration. Often, the patient has been drinking extreme amounts of sugar-containing drinks, leading to a vicious circle in regard to the water loss. A number of skin rashes can occur in diabetes that is collectively known as diabetic dermadromes (NHES, 2011)).

2.3.3 Causes of diabetes

The cause of diabetes depends on the type. Type 2 diabetes is due primarily to lifestyle factors and genetics. Type 1 diabetes is also partly inherited and then triggered

by certain infections, with some evidence pointing at Coxsackie B4 virus. There is a genetic element in individual susceptibility to some of these triggers which has been traced to particular human leucocyte antigen (HLA) genotypes (i.e., the genetic "self" identifiers relied upon by the immune system). However, even in those who have inherited the susceptibility, type 1 diabetes mellitus seems to require an environmental trigger (Chrouse *et al*., 2009).

2.3.4 Diagnosis of diabetes

Diabetes mellitus is characterized by recurrent or persistent hyperglycemia, and is diagnosed by demonstrating any one of the following (WHO, 1999). Fasting plasma glucose level ≥ 7.0 mMol/L (126 mg/dL). Plasma glucose ≥ 11.1 mMol/L (200 mg/dL) two hours after a 75 g oral glucose load as in a glucose tolerance test. Symptoms of hyperglycemia and casual plasma glucose ≥ 11.1 mMol/L (200 mg/dL). Glycated hemoglobin (Hb A1C) ≥ 6.5%.

A positive result, in the absence of unequivocal hyperglycemia, should be confirmed by a repeat of any of the above-listed methods on a different day. It is preferable to measure a fasting glucose level because of the ease of measurement and the considerable time commitment of formal glucose tolerance testing, which takes two hours to complete and offers no prognostic advantage over the fasting test (Saydah *et al*., 2001). According to the current definition, two fasting glucose measurements above 126 mg/dL (7.0 mMol/L) is considered diagnostic for diabetes mellitus. People with fasting glucose levels from 100 to 125 mg/dL (5.6 to 6.9 mMol/L) are considered to have impaired fasting glucose. Patients with plasma glucose at or above 140 mg/dL (7.8 mMol/L), but not over 200 mg/dL (11.1 mMol/L), two hours after a 75 g oral glucose load are considered to have impaired glucose tolerance. Of these two pre-diabetic states, the latter in particular is a major risk factor for progression to full-blown diabetes mellitus as well as cardiovascular disease (Baynes, 2009).

2.3.5 Management of diabetes

Diabetes mellitus is a chronic disease which is difficult to cure. Management concentrates on keeping blood sugar levels as close to normal ("euglycemia") as possible without presenting undue patient danger. This can usually be with close dietary management, exercise, and use of appropriate medications (insulin only in the case of type 1 diabetes mellitus. Oral medications may be used in the case of type 2 diabetes, as well as insulin) (Baynes, 2009).

Patient education, understanding, and participation is vital since the complications of diabetes are far less common and less severe in people who have well-managed blood sugar levels (Nathan *et al*., 2005) .Wider health problems may accelerate the deleterious effects of diabetes. These include smoking, elevated cholesterol levels, obesity, high blood pressure, and lack of regular exercise. There are roles for patient education, diabetic support, sensible exercise, with the goal of keeping both short-term and long-term blood glucose levels within acceptable bounds. In addition, given the associated higher risks of cardiovascular disease, lifestyle modifications are recommended to control blood pressure (Adler *et al*., 2000). Oral medications using Anti-diabetic drug, Insulin therapy. Routine use of aspirin has not been found to improve outcomes in uncomplicated diabetes (Pignone *et al*., 2010).

2.4 Free radicals, reactive oxygen and nitrogen and nitrogen species

A free radical can be defined as a molecule or molecular fragment containing one or more unpaired electrons in its outermost atomic or molecular orbit and are capable of independent existence (Halliwell and Guteridge, 1999). Free radicals are atoms or groups of atoms with an odd (unpaired) number of electrons and can be formed when oxygen interacts with certain molecules. Once formed these highly reactive radicals can start a chain reaction, like dominoes. Their chief danger comes from the damage they can do when they react with important cellular components such as DNA, or the cell membrane. Cells may function poorly or die if this occurs. To prevent free radical damage the body has a defense system of antioxidants (Acworth and Bailey, 1997). Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are describes free

radical and other non-radical reactive derivatives. The reactivity of radicals is generally stronger than non-radical species though are less stable (Pham-Huy, *et al.,* 2008). Free radicals are formed from molecules by the hemolytic cleavage of a chemical bond and via redox reactions, once form these highly reactive radicals can start a chain reaction (Bahorun, *et al*., 2006 ; Valko, *et al.,* 2006).

ROS and RNS includes radicals such as superoxide, hydroxyl, peroxyl, hydroperoxyl, alkoxyl, Peroxyl, nitric oxide and lipid peroxyl and non radicals like hydrogen peroxide, hypochlorous acid, ozone singlet oxygen, peroxynitrate, nitrous acids, dinitrogen triod capable toxide, lipid peroxide (Pham-Huy *et al.,* 2008). Non radicals are also termed as oxidants and capable to lead free radical reactions in living organisms easily. Radicals are derived from oxygen characterize as the most important class of radical species generated in living system (Valko *et al.,* 2006; Miller *et al.,*1990). At high concentrations, ROS can be important mediators of damage to cell structure, nucleic acids, lipid and proteins (Valko *et al*., 2006,). Radical is responsible for lipid peroxidation and also have the capability to decrease the activity of other antioxidant defense system enzyme such as catalase (CAT) and glutathione peroxide, it causes damage to the rib nucleotide which is required for DNA synthesis. The protonated form of superoxide is water, which is more reactive and able to cross the membrane and causes damage to tissue. hydroxyl radicals' damage is most reactive chemical species. It is a potent cytotoxic agent and able to attack every molecule found in living tissue. Hydrogen peroxide is not a radical but it produces toxicity to cell by causing DNA damage, membrane disruption and release calcium ions within cell, resulting in calcium dependent proteolytic enzyme to be activated, hypochlorous acid is produced by the enzyme myeloperoxidase in activated neutrophils and initiates the deactivated of ant proteases and activation of latent protease leading to tissue damage (Halliwell and Guteridge, 1999). It has ability to damage biomolecules, directly and also decomposes to liberate toxic chlorine. Metal induced generation of ROS attack DNA and other cellular components involving polyunsaturated fatty acid residue of phospholipids, which are extremely sensitive to oxidation (Siems, 1995). Peroxyl

radicals cause damage after rearranged via a cyclisation reaction to end peroxides. Studies show that free radicals produce oxidation of the side chains of all amino acid residue of proteins, particularly cysteine and methionine (Valko, *et al*., 2007; Stadtman, 2004). The free radical produced in cells include hydrogen peroxide, hypochlorous acid, and free radicals such as the hydroxyl radical and the superoxide anion (Valko, 2006). The hydroxyl radical is particularly unstable and will react rapidly and non-specifically with most biological molecules. This species is produced from hydrogen peroxide in metal-catalyzed redox reactiotns such as the Fenton reaction (Stohs and Bagchi, 1995). These oxidants can damage cells by starting chemical chain reactions such as lipid peroxidation, or by oxidizing DNA or proteins (Sies, 1997). Damage to DNA can cause mutations and possibly cancer, if not reversed by DNA repair mechanisms (Nakabeppo *et al*., 2006; Valko *et al.*, 2004), while damage to proteins causes enzyme inhibition, denaturation and protein degradation (Standtman,1992).

The use of oxygen as part of the process for generating metabolic energy produces reactive oxygen species (Raha and Robinson, 2000). In this process, the superoxide anion is produced as a by-product of several steps in the electron transport chain (Lenaz, 2001). Particularly important is the reduction of coenzyme Q in complex III, since a highly reactive free radical is formed as an intermediate ($Q^{\cdot -}$). This unstable intermediate can lead to electron "leakage", when electrons jump directly to oxygen and form the superoxide anion, instead of moving through the normal series of well-controlled reactions of the electron transport chain (Finket *et al.*, 2000). Peroxide is also produced from the oxidation of reduced flavoproteins, such as complex I (Hirsch, 1999). However, although these enzymes can produce oxidants, the relative importance of the electron transfer chain to other processes that generate peroxide is unclear (Seaver and Imlay, 2004; Imlay and James). In plants, algae, and cyanobacteria, reactive oxygen species are also produced during photosynthesis (Demming and Adam, 2002), particularly under conditions of high light intensity (Krieger, 2004). This effect is partly offset by the involvement of carotenoids in photoinhibition, and in algae and cyanobacteria, by large amount of iodide and selenium (Kupper *et al.*, 2008), which

involves these antioxidants reacting with over-reduced forms of the photosynthetic reaction centres to prevent the production of reactive oxygen species (Szabo *et al.*, 2005).

2.4.1 Peroxidation

Polyunsaturated fatty acids (PUFAs) are abundant in cellular membranes and in low-density lipoproteins (LDL) (Dekkers, *et al*., 1996). The PUFAs allow for fluidity of cellular membranes. A free radical prefers to steal electrons from the lipid membrane of a cell, initiating a free radical attack on the cell known as lipid peroxidation. Reactive oxygen species target the carbon-carbon double bond of polyunsaturated fatty acids. The double bond on the carbon weakens the carbon-hydrogen bond allowing for easy dissociation of the hydrogen by a free radical. A free radical will steal the single electron from the hydrogen associated with the carbon at the double bond. In turn this leaves the carbon with an unpaired electron and hence becomes a free radical. In an effort to stabilize the carbon-centered free radical molecular rearrangement occurs. The newly arranged molecule is called a conjugated diene (CD). The CD then very easily reacts with oxygen to form a proxy radical. The proxy radical steals an electron from another lipid molecule in a process called propagation. This process then continues in a chain reaction (Halliwell and Gutteridge, 1999).

2.5 Antioxidant

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid, or polyphenols (Sies, 1997).

Although oxidation reactions are crucial for life, they can also be damaging; plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, vitamin A, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Insufficient levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cells. As oxidative stress appears to be an important part of many human diseases, the use of antioxidants in pharmacology is intensively studied, particularly as treatments for stroke and neurodegenerative diseases. Moreover, oxidative stress is both the cause and the consequence of disease.

Antioxidants are widely used in dietary supplements and have been investigated for the prevention of diseases such as cancer, coronary heart disease and even altitude sickness. Although initial studies suggested that antioxidant supplements might promote health, later large clinical trials with a limited number of antioxidants detected no benefit and even suggested that excess supplementation with certain putative antioxidants may be harmful (Jha *et al.,* 1995; Bjelakovic *et al.,* 2007, Baillie *et al.,* 2009;). Antioxidants also have many industrial uses, such as preservatives in food and cosmetics and to prevent the degradation of rubber and gasoline.

As part of their adaptation from marine life, terrestrial plants began producing non-marine antioxidants such as ascorbic acid (Vitamin C), polyphenols and tocopherols. The evolution of angiosperm plants between 50 and 200 million years ago resulted in the development of many antioxidant pigments – particularly during the Jurassic period – as chemical defences against reactive oxygen species that are byproducts of photosynthesis (Benzie, 2003).

Early research on the role of antioxidants in biology focused on their use in preventing the oxidation of unsaturated fats, which is the cause of rancidity (German, 1999). Antioxidant activity could be measured simply by placing the fat in a closed container with oxygen and measuring the rate of oxygen consumption. However, it was the identification of vitamins A, C, and E as antioxidants that revolutionized the field and led to the realization of the importance of antioxidants in the biochemistry of living organisms (Jacob,1999; Knight, 1998). Research into how vitamin E prevents the process

of lipid peroxidation led to the identification of antioxidants as reducing agents that prevent oxidative reactions, often by scavenging reactive oxygen species before they can damage cells (Wolf, 2005).

2.5.1 Potential of antioxidant supplements to damage health

Some antioxidant supplements may promote disease and increase mortality in humans (Ristow and Zarse, 2010; Bjelakovic *et al.,* 2007). Hypothetically, free radicals induce an endogenous response which protects against exogenous radicals (and possibly other toxic compounds) (Schulz *et al.,* 2007). Recent experimental evidence strongly suggests that this is indeed the case, and that such induction of endogenous free radical production extends the life span of Caenorhabditis elegans (Christen, 2000). Most importantly, this induction of life span is prevented by antioxidants, providing direct evidence that toxic radicals may mitohormetically exert life extending and health promoting effects (Ristow and Zarse 2010; Bjelakovic *et al*., 2007).

2.5.2 Oxidative stress in diabetes and hypertension

Oxidative stress is implicated in the pathogenesis and \or complication of diabetes and hypertension. A combination these two diseases disorders increases the risk of developing cardiovascular events (Omotayo *et al.,* 2010). The imbalance between protective antioxidant (antioxidant defense) and peroxidants is known as oxidative stress. An example of this is oxidation of membrane bound lipids and lipoproteins known as lipids peroxidation (Glutteridae, 1995). Oxidation of circulating low density lipoprotein (LDL) has been linked to the initiation and progression of atherosclerosis and ultimately to the pathogenesis of cardiovascular disease (Steinbera *et al.,* 1989). Oxidative stress has been implicated in many diseases associated with ageing (Ames *et al.,* 2003). And in the ageing process (Pryor, 2007). Recent demonstration shows that advance age, in the absence of disease, is associated with decreased antioxidant defense and increase oxidative damage (Nuhill *et al.,* 1998). The degree of oxidative stress was accentuated in chronic illness, particularly in increasing severity or an acute event was serious enough to require hospital admission (Russo *et al.,* 2012). One possible explanation for this is that

the oxidative stress may be a contributory factor (Russo *et al*., 2012). It is well established that there is an increased production of damaging free radicals in diabetes mellitus and hypertensive patients that may be due to auto oxidation of glucose and glycosylated proteins (Corella *et al.,* 2001).

In hypertensive patients, the ratio of oxidant to reduced glutathione was significantly higher, and activities of glutathione peroxidase were significantly low. Increase oxidative stress and impaired antioxidant defense mechanism are believed to be the important factors contributing to the pathogenesis and progression of diabetes and hypertension (Xu *et al.,* 2006). It is also known that person with both hypertension and diabetes mellitus are at a higher risk of developing cardiovascular events than are individuals with either risk factor also (Giualiano *et al.,* 1995). Similarly, in animal models, chemical induced diabetes produces more profound effects in spontaneously hypertensive rats (SHR) than it does in normotensive strains such as wistar-kyoto (WKY) rats (Fein *et al.,* 1984). In diabetes mellitus and hypertension the kidney is either a target or it plays a prominent role in this disorder (Laight *et al.,* 2007). The actual mechanism by which diabetes exacerbates hypertension still remains unclear. However a number of hypotheses have been postulated to explain some of these mechanisms (Surekha *et al*., 2007). One of such mechanism is the role of oxygen-derived free radicals. During atherogenesis, enhance monocyte adherence to the endothelium believed to be mediated by induced expression of adhesion molecules and chemotactic proteins is linked to imbalances or changes in the redox or oxidatives state of the endothelial cells (Turner, 1998). In diabetes, hyperglycemia and hyperlipidemia is implicated in the increase generation of reactive oxygen species from vascular cells (Ohara *et al.,* 1993).

2.5.3 Antioxidant defense in diabetes and hypertension

A number of antioxidant are involved in maintaining defenses against oxidative stress (Jung *et al.,* 2004).These mechanism vary in different intracellular compartment and comprise enzymatic and non enzymatic antioxidants. The enzymatic are superoxide dismutase (SOD), catalase and glutathione peroxidase. Non enzymatic antioxidants

include endogenous ascorbic acid (Vitamin C), alpha tocophenol (Vitamin E), glutathione and exogenous carotenoids and flavonoids (Simon, 1992).

Under normal condition there is a balance between both the activities and the intracellular levels of these antioxidants. Over production of reactive oxygen species depletes both enzymatic and non enzymatic antioxidants leading to additional reactive oxygen species accumulation and cellular damage (Radi *et al.,* 1991). Ascorbate is reported to have anti-hypertensive effects (Akpaffrong and Taylor, 1998). A number of studies have shown a negative correlation between blood pressure and ascorbate Marchioli, 1999). Ascorbate might influence blood pressure by several mechanisms, including free radical scavenging action preventing protacyclin synthase inhibition (Simon, 1992). Moreover, as the superoxide radical reacts rapidly with nitric oxide generating the oxidant peroxynitrite (Radi *et al.,* 1991). Ascorbate could increase nitric oxide bioavailability by reacting with superoxide radicals and consequently preserving the vasodilatory action of nitric oxide. Reaction of ascorbate with free radicals could stop several pathways that contribute to the maintenance of hypertension resulting in an anti-hypertensive effect (Radi *et al.,* 1991; Marchioli 1999). Hana *et al*, (2011) also reported that, after 3 week of garlic treatment, the antioxidant levels in hypertensive rats I ncreased to two-third of the normal levels. He said the increases in antioxidant levels occurred simultaneously with 50 percent decreased in both pressure to (100 ± 22mm) systolic hypertensive rats. And also garlic-treated diabetes rat after 3 weeks of garlic treatment show significant increase in antioxidant as compared to diabetic control rats.

In rodents, antioxidant agents such as tempol apocynin, butylated hydroxytoluene, and vitamin E and C show remarkable effect in preventing both oxidation of protein and lipid in cardiovascular disease (Trujillo *et al.,* 2008). The same can be said of transgenic mouse models in which NADPH oxidase components are genetically eliminated (Jung *et al.*, 2004). Recent study showed that atorvastatin can decrease cyclooxygenase-2 (Cox2)-dependent 8-isoprostane generation which causes endothelial dysfunction in rat (Virdis *et al*., 2009). Atovastation also restored nitrate oxide (NO) bioavailability by increasing expression of inducible NO1 syntethase levels and decreasing vascular NADPH oxidase-driven oxygen production (Virdis *et al.,* 2009). Total protein antagonists (Zuccollo *et al.,*

2005) and polyphenols (Wang *et al.,* 2001) are two additional examples of therapeutic agents that provide anti-inflammatory effects and vascular protection accompanied by significant improvement in protein oxidation, at least in rodent models (Kukadome *et al.,* 2006.)

2.5.4 Antioxidants and hypertension

Hypertension is a major health problem world wide. Individuals with hypertension are at an increased risk for stroke, heart disease and kidney failure. Although the etiology of essential hypertension has a genetic component, lifestyle factors such as diet play an important role. More than 600 million people suffer from hypertension world wide. Insulin resistance and glucose intolerance are common features of hypertension in human and in animal models. Altered glucose metabolism leads to an increase production of the reactive aldehyde, methylglyoxal. Methylglyoxal binds sulfurhydryl and amino groups of proteins forming conjugates/advanced glycated end products (AGEs) which trigger atherosclerotic process. Prolong exposure to hyperglycemia also lead to the increase oxidative stress. This alterations impaired endothelial function leading to an increase in intracellular free calcium, peripheral vascular resistance, and hypertension. Supplementation with antioxidants including vitamin C, E, or B_6 thiols, such as lipolic acid and cysteine and quinine enzymes Q10 have been shown to lower blood pressure in animal models and humans with essential hypertension. The dietary approaches to stop hypertension (DASH) studies demonstrated that a well balanced diet rich in these nutrients was effective in lowering blood pressure. These antioxidants may achieve their hypertensive effect by reducing aldehyde conjugate/AGE formation and oxidative stress, improving insulin resistance and endothelial function, or by normalizing calcium channels and peripheral vascular resistance. In essential hypertension deficiency of antioxidant may exist or a higher than normal amount may be required to correct metabolic abnormalities. Dietary supplementation with antioxidants may be a beneficial, inexpensive, first alternate treatment mortality for hypertension (Sudesh *et al.,* 2005).

2.6 Lipid profiles in diabetes and hypertension

Lipid abnormalities are commonly found in person with diabetes and hypertension (Martorell, 2010). In Nigeria, diabetes and hypertension are noted to occur in 2 – 4% and 10–15% of population respectively (Akinkugbe, 2009). Lipid profiles abnormalities are commonly found in person with diabetes and hypertension. Diabetes which co-exist with obesity and dyslipidemia is common in diabetes mellitus (DM) as both insulin deficiency and resistance affects enzymes and pathways of lipid metabolism (Gordon *et al.*, 2010) which characterized by high triglyceride, decreased high density lipoproteins (HDL), increased total cholesterol and low density lipoproteins (LDL) (Jimoh *et al.,* 2010). Lipoprotein lipase is the main enzyme for the catabolism of chylomicrons and very low density lipoprotein (VLDL) particles which enable them to form remnants that are cleared by apoliprotein E (APO E) and apoliprotein B (APO B) receptors in the liver. Lipoprotein lipase is an insulin dependent enzyme and resistance will lead to increase triglyceride levels metabolism of high density lipoprotein (HDL) cholesterol in type 2 diabetes mellitus (DM) is not well understood but it's clearance is thought to be mediated by VLDL receptors which are mainly located in the liver (Gordon *et al.,* 2010; Orchard, 1990). Insulin has been said to play a role in regulation of VLDL receptor binding and internalization leading to a decrease in HDL catabolism. Orchard, (1990) proposed that there is an APO Blood in patients with type 2 DM which then causes a reduction in the production of HDL and thereby compensating for reduced clearance. There is an inverse relationship between VLDL clearance and HDL-cholesterol, thus there is a decrease in HDL concentration in type 2 DM. HDL cholesterol levels may also be further reduced in Type 2 DM due to elevated hepatic lipase activity which catabolizes HDL-cholesterol.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

Centrifuge-UNISCOPE SM9053; Surgifriend Medicals, England, Optima SP-300 Spectrophotometer made in Japan, Haier Thermocool model BD -198 effective volume 198L, Memmert Incubator made in China.

3.2 Sample selection

Biochemical investigations were carried out in the blood samples of 200 subjects. The study location was Yola, Adamawa State, Nigeria. A total of 200 samples were taken to assess for fasting blood sugar, total antioxidant, lipid peroxides and lipid profile. 50 confirmed diabetes, 50 confirmed hypertensive, 50 that were both diabetic and hypertensive patients and another 50 samples of normal individuals which served as control groups. Age groups in both diabetic, hypertensive and control were taken from 20-55 years. All the subjects were then divided according to their age groups. The samples were analyzed for fasting blood sugar, total antioxidant, lipid peroxidation and lipid profiles.

3.3 Methods

In all subjects 10ml blood samples were collected in Na-EDTA (1mg/ml) tubes after a fasting period of 10-12 hours. The plasma samples obtained by centrifuging the whole blood in a UNISCOPE SM9053 centrifuge, England. It was centrifuged at 2500g for 5 minutes. In all the samples, total antioxidant, lipid peroxidation and lipid profiles were assayed immediately.

3.3.1 Determination of glucose

Randox-assay kit (CAT.NO GLU 364) method based on Barham and Trinder (1972) was used. The principle involves the enzymatic oxidation of glucose in sample by the enzyme glucose oxidase which generates hydrogen peroxide (H_2O_2) and gluconic

acid. The concentration of hydrogen peroxide released is proportional to initial amount of glucose in the sample and it reacts under catalysis of peroxidase, with phenol and 4-amino phenazone to form a red violet quinoneimine dye whose colour intensity reflects the concentration of glucose in the sample.

Procedure: Chemically cleaned test-tubes were arranged in test-tube rack and labeled blank, standard and sample, to blank test-tube 20µl of distil water were added, to standard test-tube 20µl standard reagent were added and to sample test-tubes 20 µl of the plasma samples were also added, then 20µl of glucose reagent were added to all the test-tube respectively, were mixed and incubated for 5mins at 37^0C the absorbance (ABS) standard (Sb TD) and sample were measured against the reagent blank at 510nm.

Calculation: concentration of glucose in mmol/l was obtained as follows;

$$\text{Concentration of glucose in the sample} = \frac{\text{O. D of test sample}}{\text{O. D of standard}} \times \text{concentration of standard}$$

3.3.2 Determination of total antioxidant (liquid stable) enzymatic colorimetric method

Determination of total antioxidant was carried out using BIOREX Kit (BXCO553A) based on the method of Halliwell, (2000). The principle is based on the absorbance of 1, 2 – Azino-di-3-ethylbenzthiazoline sulphonate when incubated with a peroxidase (metmyoglobin) and H_2O_2 to produce the radical cation absorbance. This has a stable blue green colour which is measured at 600-660nm. Antioxidant in the sample, suppressed the formation of this color to a degree which is proportional to their concentration.

Procedure: Chemically cleaned teat-tubes were arranged in test-tube rack labeled blank, standard and sample, to blank test-tube 50µl of distil water were added, then 50µl standard reagent were added to standard test-tube and 50µl of test sample were added to sample test-tubes, 800µl of total antioxidant status (TAS) buffer were added to blank, standard and sample respectively and the initial absorbance was measured at 660nm against the reagent blank which was written as initial absorbance, then 125µl of TAS. chromogen were added to blank, standard and sample mixture respectively, they were

mixed and incubated for 5min at 37^0C and was read at 660nm wavelength which was final absorbance.

Calculation: Absorbance sample = (final absorbance of sample – initial absorbance of sample) = O.D of test sample

Absorbance standard = (final absorbance of standard – initial absorbance of standard) = O.D of standard.

Results in µmol/L:

$$\text{Conc of total antioxidant in test sample} = \frac{\text{O. D of test sample}}{\text{O. D of standard}} \times \text{Conc of standard}$$

3.3.3 Determination of lipid peroxide

Determination of lipid peroxides was carried out using BIOREX Kit (BXCO571A) which was based on the method of (Gavino *et al*., 1981) and the principle is based on the formation of malondialdehyde (MDA) an end product of lipid peroxidation which reacts with TBA (Thiobarbituric acid to form a faint pink colored product).

Procedure: About 0.5ml of plasma, 0.5ml of normal saline was added and an equal volume of trichloroacetic acid (TCA) were added in a cleaned test-tube and incubated at 37°C for 20 minutes and centrifuged at 500g. To 1ml of TCA extract (the supernatant), 0.25ml TBA was added and heated in a water bath at 95°C for 1hour till a faint pink color appeared. After cooling the color was extracted in 1ml butanol and the intensity was read at 532nm using shimadu UV-240 spectrophotometer and 1,1,3,3 tetra ethoxypropane (1-10mmol/ml) was used as the standard.

Calculation: Concentration of lipid peroxide in test sample in MMol/L= 8412 x Absorbance of sample.

3.3.4 Determination of total cholesterol

Determination of total cholesterol was carried out using Randox kit (CAT. NO.HN 203) which was based on the endpoint, increasing reaction, method of Friedwald, (1972) and the principle is based on the enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the

presence of phenol and peroxidase. Absorbance will be measured colorimetrically at 500nm.

Procedure: Sterile test-tubes were arranged in a test-rack and were labeled blank, standard and sample, to blank test-tube 10µl of distil water was added, 10µl of standard reagent was added to standard test-tube and10µl of sample was added to the sample test-tube and then 100µl of cholesterol reagent was added to the respective test-tubes and mixed. The mixtures were incubated for 10 minutes at 25°C, the absorbance of the samples were measured against the reagent blank within 60 minutes.

Calculation:

$$\text{Conc of cholesterol in the sample} = \frac{\text{Abs of sample}}{\text{Abs of standard}} \times \text{Conc of std (mMol/L)}$$

3.3.5 Determination of triglycerides

Determination of triglycerides was carried out using Randox kit (CAT. NO. TR210) which was based on the method of Friedwald, (1972) and the principle is based on the enzymatic hydrolysis with lipases. The indicator is a 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase.

Procedure: Sterile test-tubes were arranged in a test-tube rack labeled blank, standard and sample, 10µl of distil water was added to blank test-tube, 10µl of standard reagent was added to the standard test-tube and 10µl of the test sample was added to the sample test-tube. 1000µl of reagent (R1) was added to the blank, standard and sample test tubes. The mixture was incubated for 5min at 37^{o}C. The absorbance of the sample and standard were measured against the reagent blank at 546nm wavelength within 60 minutes.

Calculation:

$$\text{Conc of triglycerides in test sample} = \frac{\text{Abs of test sample}}{\text{Abs of standard}} \times \text{Conc of std (mM/L)}$$

3.3.6 Determination of high density lipoproteins

Determination of high density lipoprotients (HDL). Cholesterol was carried out using Randox Kit (CAT. NO. CH 203) based on the principle of low density lipoproteins (LDL and VLDL) chylomicron fractions are precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the

cholesterol concentration in the HDL (high density lipoprotein) fraction, which remains in the supernatant, is determined.

Procedure: Chemically cleaned test-tube were arranged in a test-tube rack and labeled blank, standard and sample. First step, 500µl of standard reagent were added to standard test-tube and 500µl of sample were added sample test-tube, and 1000µl of precipitant phosphotungstic (R1) were added to standard and sample respectively and were mixed, allowed to stand for 10 minutes at room temperature and centrifuged for 2 minutes at 12,000 rpm. Second step, to blank test-tube10µl distil were added, 10µl clear supernatant of standard and sample were added to each of the test-tube respectively and 1000µl of cholesterol reagent were also added to all the test-tube respectively and were mixed, incubated for 5 minutes at 37^{o}C. The sample and standard were measured against reagent blank within 60 minutes.

Calculation

$$\text{Conc of HDL in sample} = \frac{\text{Abs of sample}}{\text{Abs of std !}} \times \text{conc. of std}$$

3.3.7 Determination of low density lipoprotein

The concentration of LDL was calculated as described by Friedewald *et al*., 1972.

$$\text{Conc of LDL} = \frac{\text{Conc of total cholesterol in the sample} - \text{conc of triglyceride}}{2.2 \; - \; \text{conc of HDL cholesterol in the sample}}$$

3.4 Statistical Analysis

Mean ± SD, in total antioxidant and lipid peroxidation and lipid profile were determined among age groups for both male and female in non diabetic control and debetic and hypertension. T-test was use to compare between the non diabetic and debetic group/hypertension. Significant level was determined at $p<0.05$ confidence level

CHAPTER FOUR

RESULTS

Biochemical investigations were carried out on the blood specimens of 50 diabetic, 50 hypertensive, 50 diabetic- hypertensive patients, and 50 apparently healthy individuals were used as control subjects. Age and sex were used to match patients with control individuals. The levels of fasting blood glucose, total antioxidant status, lipid peroxidation and lipid profile were estimated in the patients and control subjects.

The biochemical parameters of diabetic patients and control subjects are shown on table 4.1. The levels of glucose (14.51 ± 2.32), lipid peroxide (0.71 ± 0.57), total cholesterol (5.51 ± 3.66), and LDL (3.67 ± 1.85) were significantly increased ($p<0.05$) in diabetic patients compared to control subjects which were, glucose (4.46 ± 0.80), lipid peroxide (1.84 ± 0.52), total cholesterol (2.73 ± 0.71), and LDL (1.18 ± 0.52) respectively. While the levels of total antioxidant (0.71 ± 0.57) and HDL (0.09 ± 0.63) were significantly decreased ($p<0.05$) compared to control subjects which had total antioxidant (2.02 ± 0.40) and HDL (1.80 ± 0.44) respectively.

TABLE 4.1: Biochemical parameters of diabetic patients and control subjects

Parameters (mmol/L)	**Subjects**	
	Diabetic patients	Control
Glucose	14.51 ± 2.32[a]	4.46 ± 0.80
Total antioxidant	0.71 ± 0.57[b]	2.02 ± 0.40
Lipid peroxide	5.51 ± 3.66[a]	1.84 ± 0.52
Total Cholesterol	4.55 ± 1.21[a]	2.73 ± 0.71
Triglyceride	1.49 ± 0.93	1.62 ± 0.75
HDL	0.09 ± 0.63[b]	1.80 ± 0.44
LDL	3.67 ± 1.85[a]	1.18 ± 0.52

Results are Mean SD; n=50
a = Values are significantly ($P<0.05$) higher than those of control subjects
b = values are significantly ($P<0.05$) lower than those of control subjects.

The biochemical parameters of hypertensive patients and control subjects are shown in table 4.2. The levels of lipid peroxide (10.95 ± 5.15), total cholesterol (5.37 ± 1.15), and LDL (4.52 ± 1.75) were significantly increased ($p<0.05$) in hypertensive patients compared to control subjects which were, lipid peroxide (1.84 ±0.52), total cholesterol (2.73 ± 0.71), and LDL (1.18 ± 0.52) respectively. The levels of total antioxidant (0.77 ± 0.35) and HDL (0.72 ± 0.42) were significantly decreased ($p<0.05$) compared to control subjects which had total antioxidant (2.02 ± 0.40) and HDL (0.80 ± 0.44) respectively.

TABLE 4.2: Biochemical parameters of hypertensive patients and control subjects

Parameters (mmol/L)	**Subjects**	
	Hypertensive patients	Control
Glucose	4.36 ± 0.64	4.46 ± 0.80
Total Anti Oxidant	0.77 ± 0.35[b]	2.02 ± 0.40
Lipid peroxide	10.95 ± 5.15[a]	1.84 ± 0.52
Total Cholesterol	5.37 ± 1.15[a]	2.73 ± 0.71
Triglyceride	1.40 ± 0.79	1.62 ± 0.75
HDL	0.72 ± 0.42[b]	0.80 ± 0.44
LDL	4.52 ± 1.75[a]	1.18 ± 0.52

Results are Mean X ± SD; n = 50
a= values are significantly (P<0.05) higher than those of control subjects
b= values are significantly (P<0.05) lower than those control subjects.

The biochemical parameters of diabetic/hypertensive patients and control subjects are shown in table 4.3. The levels of glucose (16.95 ± 2.71), lipid peroxide (10.77 ± 3.21), total cholesterol (5.53 ± 0.87), and LDL (4.80 ± 2.06), were significantly increased ($p<0.05$) in diabetic/hypertensive patients compared to control subjects which were (4.46 ± 0.80), lipid peroxide (1.84 ± 0.52), total cholesterol (2.73 ± 0.71) and LDL (1.18 ± 0.52). While the levels of total antioxidant (0.57 ± 0.45) and HDL (0.73 ± 0.43), were significantly decreased ($p<0.05$) compared to the values of control subjects which had total antioxidant (2.02 ± 0.40) and HDL (1.18 ± 0.52) respectively.

TABLE 4.3: Biochemical parameters of hypertensive-diabetics patients and control subjects

Parameters (mmol/L)	**Subjects**	
	Hypertensive/Diabetic patients	Control
Glucose	16.95 ± 2.71	4.46 ± 0.80
Total Anti Oxidant	0.5 ± 0.45[b]	2.02 ± 0.40
Lipid peroxide	10.77 ± 3.21[a]	1.84 ± 0.52
Total Cholesterol	5.37 ± 0.52[a]	2.73 ± 0.71
Triglyceride	1.49 ± 0.52	1.62 ± 0.75
HDL	0.73 ± 0.43[b]	0.80 ± 0.44
LDL	4.80 ± 2.06[a]	1.18 ± 0.52

Results are Mean X ± SD; n = 50
a= values are significantly (P<0.05) higher than those of control subjects
b= values are significantly (P<0.05) lower than those control subject

The biochemical parameters using age groups of diabetic patients are shown on table 4.4. The levels of glucose (16.15 ± 2.19), lipid peroxide (6.2 ± 13.84) and LDL (4.24 ± 1.67) of 36-55 years significantly increased ($p<0.05$) compared to the values of 20-35years which were glucose (12.23 ± 0.85), lipid peroxide (3.04 ± 1.00) total cholesterol (4.05 ± 0.91) and LDL (1.66 ± 0.63) respectively. While the levels of total antioxidant (0.48 ± 0.40) and HDL (0.88 ± 0.39) of 36-55 years were significantly decreased ($p<0.05$) compared to the values 20-35 years which had total antioxidant (1.52 ± 0.26) and HDL (1.86 ± 0.71) respectively.

TABLE 4.4: Biochemical parameters using age groups of diabetic patients

Parameters (mmol/L)	**Age group of Subjects**	
	20-35	36-55
Glucose	12.23 ± 0.85	16.15 ± 2.19[a]
Total Anti Oxidant	1.52 ± 0.26	0.48 ± 0.40[b]
Lipid peroxide	3.04 ± 1.00	6.21 ± 3.84[a]
Total Cholesterol	4.05 ± 0.91	4.50 ± 1.25
Triglyceride	1.24 ± 0.51	1.56 ± 1.01
HDL	1.86 ± 0.71	0.88 ± 0.39[b]
LDL	1.66 ± 0.63	4.24 ± 1.67[a]

Results are Mean ± SD n=50
a= values are significantly ($P<0.05$) higher than those of 20-35 years
b= values are significantly ($p<0.05$) lower than those of 20-35 years

The biochemical parameters using age groups of hypertensive patients are shown on table 4.5. The levels of glucose (4.47 ± 0.57), lipid peroxide (11.54 ± 4.93), total cholesterol (5.51 ± 1.15) and LDL (4.75 ± 1.74) were significantly increased ($p<0.05$) compared to the values of 20-35 years which were glucose (3.60 ± 0.57), lipid peroxide (6.57 ± 4.99), total cholesterol (4.35 ± 0.36) and LDL (2.85 ± 0.45) respectively. While the level of total antioxidant (0.68 ± 0.25) were significantly decreased ($p<0.05$) compared to the value of 20-35 years which had total antioxidant (1.42 ± 0.26).

TABLE 4.5: Biochemical parameters using age groups of hypertensive patients

Parameters (mmol/L)	**Subjects**	
	20-35	36-55
Glucose	3.60 ± 0.57	4.47 ± 0.57[a]
Total Anti Oxidant	1.42 ± 0.26	0.68 ± 0.25[b]
Lipid peroxide	6.57 ± 4.99	11.54 ± 4.93[a]
Total Cholesterol	4.35 ± 0.36	5.51 ± 1.15[a]
Triglyceride	1.25 ± 0.59	1.42 ± 0.82
HDL	0.92 ± 0.31	0. 69 ± .0.43
LDL	2.85 ± 0.45	4.75 ± 1.74[a]

Results are Mean ± SD n=50
a=values are significantly (P<0.05) higher than those of 20-35 years
b= values are significantly (P<0.05) lower than those of 20-35 years

The biochemical parameters using age groups of diabetics /hypertensive patients are shown on table 4.6. The levels of glucose (16.68 ± 2.66), lipid peroxide (11.69 ± 2.67), total cholesterol (5.72 ± 0.77) and LDL (5.23 ± 2.16) were significantly increased ($p<0.05$) compared to the values of 20-35 years which were glucose (12.62 ± 1.05), lipid peroxide (7.84 ± 3.08), total cholesterol (4.87 ± 0.87) and LDL (3.44 ± 0.64). While the values of total antioxidant (0.38 ± 0.22) and HDL (0.66 ± 0.43) were significantly decreased ($p<0.05$) compared to values of 20-35 years which had total antioxidant (1.19 ± 0.42) and HDL (0.94 ± 0.37) respectively.

TABLE 4.6: Biochemical parameters using age groups of diabetic-hypertensive patients

Parameters (mmol/L)	**Subjects**	
	20-35	36-55
Glucose	12.62 ± 1.05	16.68 ± 2.66[a]
Total Anti Oxidant	1.19 ± 0.42	0.38 ± 0.22[b]
Lipid peroxide	7.84 ± 3.08	11.69 ± 2.67[a]
Total Cholesterol	4.89 ± 0.87	5.72 ± 0.77[a]
Triglyceride	1.24 ± 0.39	1.57 ± 0.54
HDL	0.94 ± 0.37	0.66 ± 0.43[b]
LDL	3.44 ± 0.64	5.23 ± 2.16[a]

Results are Mean ± SD n=50
a= values are significantly (P<0.05) higher than those of 20-35 years
b= values are significantly (P<0.05) lower than those of 20-35 years.

The biochemical parameters of diabetics and diabetic/ hypertensive patients are shown on table 4.7. The levels of lipid peroxide (10.77 ± 3.21) and LDL (4.80 ± 2.06), were significantly increased ($P<0.05$) in diabetic / hypertensive patients compared to the values of diabetic patients which had lipid peroxide (5.51 ± 3.66) and LDL (3.67 ± 1.85) respectively. While the levels of total antioxidant (0.57 ± 0.4) and HDL (0.73 ± 0.43) were significantly decreased ($p<0.05$) compared to values of diabetes which had total antioxidant (0.71 ± 0.57) and HDL (1.09 ± 0.63) respectively.

TABLE 4.7: Biochemical parameters of diabetic and diabetic-hypertensive patient

Parameters (mmol/L)	Diabetic	Diabetic + Hypertensive
Glucose	14.51 ± 2.32	16.95 ± 2.71
Total Anti Oxidant	0.71 ± 0.57	0.57 ± 0.4[b]
Lipid peroxide	5.51 ± 3.66	10.77 ± 3.21[a]
Total Cholesterol	4.55 ± 1.21	5.53 ± 0.87
Triglyceride	1.49 ± 0.93	1.49 ± 0.52
HDL	1.09 ± 0.63	0.73 ± 0.43[b]
LDL	3.67 ± 1.85	4.80 ± 2.06[a]

Results are Mean ± SD n=50
a= values are significantly (P<0.05) higher than those of diabetes patients
b= values are significantly (P<0.05) lower than those of diabetes patients.

The biochemical parameters of hypertensive and diabetic/hypertensive patients are shown table 4.8. The levels of glucose (16.95 ± 2.71) were significantly increased ($p<0.05$) compared to the valves hypertensive patients which had glucose (4.36 ± 0.64). While the levels of total antioxidant (0.57 ± 0.45) were significantly decreased ($p<0.05$) compared to values of hypertensive patients which had total antioxidant (0.77 ± 0.35).

TABLE 4.8: Biochemical parameters of hypertensive and diabetic-hypertensive patients

Parameters (mmol/L)	Hypertensive	Diabetics+ Hypertensive
Glucose	4.36 ± 0.64	16.95 ± 2.71[a]
Total Anti Oxidant	0.77 ± 0.35	0.57 ± 0.45[b]
Lipid peroxide	10.95 ± 5.15	10.77 ± 3.21
Total Cholesterol	5.37 ± 1.15	5.53 ± 0.87
Triglyceride	1.40 ± 0.79	1.49 ± 0.52
HDL	0.72 ± 0.42	0.73 ± 0.43
LDL	4.52 ± 75	4.80 ± 2.06

Results are Mean ± SD n=50
a= values are significantly ($P<0.05$) higher than those of hypertensive patients
b= values are significantly ($P<0.05$) lower than those of hypertensive patients.

The biochemical parameters of diabetic and hypertensive patients 20-35 years age range are shown on table 4.9. The levels of glucose (14.23 ± 0.85) were significantly increased ($p>0.05$) in diabetic patients compared to hypertensive patient which had glucose values (3.60 ± 0.57).While the lipid peroxide (6.57 ± 4.99) and LDL (2.85 ± 0.63) were significantly increased ($p<0.05$) in hypertensive patients compared to the values of diabetic patients which had lipid peroxide (3.04 ± 1.00) and LDL (1.66 ± 0.63) respectively. While the levels of HDL (0.9 ± 0.31) was significantly decreased ($p<0.05$) increase in hypertensive patients compared to values of diabetics patients (1.86 ± 0.71).

TABLE 4.9: Biochemical parameters of diabetics and hypertensive patients of 20-35 years age range

Parameters (mmol/l)	Diabetics	Hypertensive
Glucose	14.23 ± 0.85[a]	3.60 ± 0.57
Total Anti Oxidant	1.52 ± 0.26	1.42 ± 0.26
Lipid peroxide	3.04 ± 1.00	6.57 ± 4.99[a]
Total Cholesterol	4.05 ± 0.91	4.35 ± 0.36
Triglyceride	1.24 ± 0.51	1.25 ± 0.59
HDL	1.86 ± 0.71	0.92 ± 0.31[b]
LDL	1.66 ± 0.63	2.85 ± 0.45[a]

Results are Mean ± SD n=50
a = values are significantly ($P<0.05$) higher than those of diabetes patient
b= values are significantly ($P<0.05$) lower than those of diabetes patients

The biochemical parameters of diabetics and hypertensive patients of 36-55 range are shown on table 4.10. The levels of glucose (16.15 ± 2.19) was significantly increased ($p<0.05$) in diabetic patients compared to the values of hypertensive patients which had glucose (4.47 ± 0.57). There was significantly increased ($p<0.05$) in lipid peroxide (11.54 ± 4.93) and total cholesterol (5.51 ± 1.15) of hypertensive patients compared to the values of diabetes patients which had lipid peroxide (6.21 ± 3.84) and total cholesterol (4.70 ± 1.25) respectively. While the levels of total antioxidant (0.48 ± 0.40) was significantly decreased ($p<0.05$) in diabetic patients compared to values of hypertensive patients which had total antioxidant (0.68 ± 0.25).

TABLE 4.10: Comparison of biochemical parameters of diabetic and hypertensive patients of 36-55 years age range

Parameter (mmol/L)	Diabetic	Hypertensive
Glucose	16.15 ± 2.19[a]	4.47 ± 0.57
Total Anti Oxidant	0.48 ± 0.40	0.68 ± 0.25[a]
Lipid peroxide	6.21 ± 3.84	11.54 ± 4.93[a]
Total Cholesterol	4.70 ± 1.25	5.51 ± 1.15[a]
Triglyceride	1.56 ± 1.01	1.42 ± 0.82
HDL	0.88 ± 0.39	0.69 ± 0.43[**b**]
LDL	4.24 ± 1.67	4.75 ± 1.74

Results are Mean ± SD n=50
a = values are significantly (P<0.05) higher than those of diabetes patients
b= valves are significant (P<0.05) lower than those diabetes patients

The correlation between glucose and total antioxidant/lipid of diabetic subject are shown on table 4.11. There was negative correlation between total antioxidant ($r = -0.866$; $p = 0.000$), and HDL ($r = -0.399$; $p = 0.004$) in glucose level of diabetic patients compared to positive correlation which were lipid peroxide ($r = 0.844$; $p = 0.000$), total cholesterol ($r = 0.624$; $p = 0.000$), triglyceride ($r = 0.366$; $p = 0.009$), and LDL ($r = 0.864$; $p = 0.000$) in glucose level of diabetic patients.

Table 4.11: Correlation between Glucose and Total anti-oxidant/Lipid profile among diabetic subjects

Parameter (mmol/L)	**Correlation coefficient (r)**	**P-value**	**Remarks**
Total Anti-Oxidant	-0.866	0.000	Significant
Lipid peroxide	0.844	0.000	Significant
Total cholesterol	0.624	0.000	Significant
Triglyceride	0.366	0.009	Significant
HDL	-0.399	0.004	Significant
LDL	0.864	0.000	Significant

Figures with negative values show negative correlation i.e less than normal values.
Figures with positive values show positive correlation i.e above normal values.

The correlation between glucose and total antioxidant/lipid of diabetic/hypertensive subject are shown on table 4.12. There was negative correlation between total antioxidant ($r = -0.563$; $p = 0.000$) and HDL ($r = -0.356$; $p = 0.011$) in glucose level of diabetic patients compared to positive correlation which were lipid peroxide ($r = 0.432$; $p = 0.002$), total cholesterol ($r = 0.351$; $p = 0.013$), triglyceride ($r = 0.164$; $p = 0.255$) and LDL ($r = 0.401$; $p = 0.000$) in glucose level of diabetic patients.

Table 4.12: Correlation between Glucose and Total anti-oxidant/Lipid profile among hypertensive-diabetic subjects

Parameter (mmol/L)	**Correlation coefficient (r)**	**P-value**	**Remarks**
Total Anti-Oxidant	-0.593	0.000	Significant
Lipid peroxide	0.432	0.002	Significant
Total cholesterol	0.351	0.013	Significant
Triglyceride	0.164	0.255	Significant
HDL	-0.356	0.011	Significant
LDL	0.401	0.000	Significant

Figures with negative values show negative correlation i.e less than normal values.
Figures with positive values show positive correlation i.e above normal values.

The correlation between total antioxidant and lipid among diabetic subject are shown on table 4.13. There was negative correlation between lipid peroxide ($r = -0.693$; $p = 0.000$), total cholesterol ($r = -0.556$; $p = 0.000$), triglyceride (-0.336; $p = 0.017$) and LDL (-0.843; $p = 0.000$) in total antioxidant level of diabetic patients compared to positive correlation which had HDL ($r = 0.519$; $p = 0.000$) in total antioxidant of diabetic patients.

Table 4.13: Correlation between Total anti-oxidant and Lipid profile among diabetic subjects

Parameter (mmol/L)	**Correlation coefficient (r)**	**P-value**	**Remarks**
Lipid peroxide	-0.693	0.000	Significant
Total cholesterol	-0.556	0.000	Significant
Triglyceride	-0.336	0.017	Significant
HDL	0.519	0.000	Significant
LDL	-0.843	0.000	Significant

Figures with negative values show negative correlation i.e less than normal values.
Figures with positive values show positive correlation i.e above normal values.

The correlation between glucose and total antioxidant/lipid of diabetic/ hypertensive subject are shown on table 4.14. There was negative correlation between lipid peroxide ($r = -0.752$; $p = 0.000$), total cholesterol ($r = -0.461$; $p = 0.001$), triglyceride ($r = -0.311$; $p = 0.028$) and LDL ($r = -0.495$; $p = 0.000$) in total antioxidant of hypertensive/diabetic subject compared to positive correlation which had HDL (0.360; $p = 0.010$) in total antioxidant of diabetic patients.

Table 4.14: Correlation between Total anti-oxidant and Lipid profile among hypertensive - diabetic subjects

Parameter (mmol/L)	**Correlation coefficient (r)**	**P-value**	**Remarks**
Lipid peroxide	-0.752	0.000	Significant
Total cholesterol	-0.461	0.001	Significant
Triglyceride	-0.311	0.028	Significant
HDL	0.360	0.010	Significant
LDL	-0.495	0.000	Significant

Figures with negative values show negative correlation i.e less than normal values.
Figures with positive values show positive correlation i.e above normal values.

CHAPTER FIVE

DISCUSSION

The worldwide morbidity of diabetes has increased rapidly even in developing countries, doubling the combined risk of cardiovascular events in patients with hypertension (Ying *et al*., 2007). Vascular disease in hypertension and diabetes is associated with increased oxidant arising from NADPH oxidase and xanthine oxidase. Among all cardiovascular risk factors, diabetes mellitus (DM) and hypertension are the leading causes of cardiovascular diseases (Richard and Cohen, 2010). Unfortunately, these two risk factors often coexist, such that 60% of the patients with diabetes are hypertensive and up to 20% of patients with hypertension are diabetic (Savoia and Schiffrin, 2006).

In this study, increase level of serum glucose in diabetic patients and diabetic-hypertensive patients where observed when compared to control. Type 1 diabetes is caused by the immune system destroying the cells in the pancreas that make insulin. This causes diabetes by leaving the body without insulin to function normally (Meng and Fong, 2008). There was significantly increased of glucose level in diabetic-hypertensive patients when compared with diabetic patients alone and hypertensive patients alone as seen in Tables 7 and 8. Increased levels of glucose found in diabetic-hypertensive patient in this study agreed with the work of Epstein and Sower (1995) which stated that "elevated circulating levels of insulin, existing in type 2 diabetes in many patients with essential hypertension may contribute directly or in conjunction with insulin-like growth factor to the accelerated atherosclerosis associated with these conditions, and this condition is as a result of two risk factors which often co-exist together" (Wassmann *et al*., 2004).

There was significant decrease in the levels of total antioxidant status of diabetics, hypertensive and diabetic-hypertensive patients when compared with values of control group. These decrease in total antioxidant level may lead to increase in circulating reactive oxygen species (ROS) resulting into high risk of oxidative damage and possibly increase in blood pressure (Ceriello, 2008). Significant decrease of total antioxidant

status in hypertensive patients is a risk factor for coronary heart disease (Corella *et al.*, 2001). This study agreed with the work of Richard and Cohen (2010) which stated that in diabetes, hyperglycemia and hyperlipidemia is implicated in the increased generation of reactive oxygen species from vascular cells. The decreased antioxidant in status diabetic-hypertensive patient is as a result of low antioxidant bioavailability which promotes cellular oxidative stress and oxidative damage associated with hypertension and diabetics (Wassmann *et al.,* 2004).

The oxidative stress may trigger a number of oxidant responsive genes that play a prominent role in monocyte–endothelia dysfunction (Laight *et al.,* 2007). Although mitochondria and xanthine oxidase are implicated as sources of damaging reactive species in diseases like diabetes and hypertension, NADPH oxidases, either in inflammatory leukocytes or vascular cells, account for the bulk production of reactive oxygen species which result in decreased antioxidant level (Meng and Fang, 2008). Increased level of lipid peroxidation was found in diabetic, hypertensive, diabetic-hypertensive patients when compared with valves for control.

The increase of lipid peroxide of diabetic patients is as a result of the oxidative stress which is presently accepted as a likely causative factor in the development of insulin resistance which is present in obesity (Dandona *et al*., 2005). The mechanisms leading to the insulin cascade down-regulation in cells subjected to oxidative stress involves increase serine/threonine phosphorylation of insulin receptor substrate-1 (IRS1), impaired insulin-stimulated, redistribution of IRS1 and phosphatidylinositol-kinase between cytosol and low-density microsomal fraction followed by a reduced protein kinase-B phosphorylation and glucose transporter4 (GLUT4) translocation to the plasma membrane.

In addition, prolonged exposure to reactive oxygen species (ROS) affects transcription of glucose transporters, sthe level of glucose transporter 1(GLUT1) is increased, GLUT4 level is reduced (Furukawa *et al*., 2004). Oxidative stress has been described as a key factor in obesity-related diseases such as diabetes, (Govindarajan *et al.,* 2005) atherosclerosis and inflammation (Scalbert *et al*., 2005). The increase in lipid peroxidation also agrees with the work of (Russo *et al.,* 2012) which stated that increase

in lipid peroxidation is initiated in polyunsaturated fatty acids in LDL surface phospholipids then propagate to core lipids resulting in oxidative modification of polyunsaturated fatty acids, cholesterol moiety and phospholipids. Hypertension is associated with increased vascular oxidative stress. Animal studies have generally supported the hypothesis that increased blood pressure is associated with increased oxidative stress (Ehud, 2008). However, human studies have been inconsistent. Oxidative stress promotes vascular smooth muscle cell proliferation and hypertrophy and collagen deposition leading to thickening of the vascular media and narrowing of the vascular lumen. In addition, increased oxidative stress may damage the endothelium and impair endothelium-dependent vascular relaxation and increases vascular contractile activity. All these effects on the vasculature explain how increased oxidative stress can cause hypertension (Mclntyre *et al.*, 1999). There was increased lipid peroxide of diabetic-hypertensive when compared with diabetic patients alone.

Recent reports have suggested that diabetic complications and hypertension have common etiology involving oxidative stress in both diabetes and hypertension which has become crucial as an index of pathology of these diseases (Hana *et al.,* 2011). A recent hypothesis point out the possible role of oxidative stress as a key player in the pathogenesis of insulin resistance, beta cell dysfunction, and hypertension (Ceriello and Motz, 2004).The increase in the level of lipid peroxide of diabetic-hypertensive subject also agrees with the findings of Bloch *et al* (2005) which also stated that in people that are obese, the leading causes of oxidative stress have been identified as hyperglycemia, insulin resistance, increased tissue lipid levels, inadequate antioxidant defenses, enzymatic sources within the endothelium and chronic inflammation.

An increase in VLDL occurs in diabetes mellitus due to increase availability of glucose for VLDL synthesis and decrease in lipoprotein lipase activity leading to decrease of VLDL from peripheral circulation. An increased percent body fat was identified with higher levels of triglyceride, and low level of HDL-C due to decrease in hepatic lipase activity resulting in decrease VLDL clearances which are metabolic abnormalities characterizing metabolic syndrome (Manu *et al.,* 2007). In diabetic patients the prolong exposure to hyperglycemia has been reported to increase oxidative stress,

level of total cholesterol and LDL cholesterol. The raised level of LDL been directly related to incidence of diabetic and coronary heart disease (Gordon *et al.,* 2010). Elevated levels of total cholesterol and LDL cholesterol in hypertensive patients confirmed the study earlier reported by (Ledwozw *et al.,* 2007). Plasma lipids profile which is altered in hypertensive patients appear to be a sign factor in the development of premature atherosclerosis, these include an increase in total cholesterol and LDL cholesterol and decreased HDL cholesterol there by resulting in disruption of membrane fluidity and leading to membrane alteration of function (Shier and Rickie. 1999). Increase in serum LDL level of diabetic-hypertensive is as a result of decreased in serum level of HDL which serve as a transport medium of LDL cholesterol. Elevated levels of triglycerides and reduced concentration of HDL-C are very common in patients with insulin resistance, leading to impaired glucose tolerance and abnormal fasting plasma glucose levels (Hans-Willi *et al.,* 2001). In Singapore, fasting serum triglyceride levels, but not HDL-C and LDL-C concentrations, were found to be higher among persons with type 2 diabetes mellitus than those of non-diabetic (Hughes *et al.,* 1998).

Increased glucose serum level of 36-55 years old diabetes patients is as a result of hyperglycemia which can induce oxidative stress, with increased in age, via several mechanisms including glucose auto oxidation, the formation of advanced glycation end-products (AGE) and activation of the polyol pathway. Other circulating factors that are elevated in diabetics such as free fatty acids and leptin also contribute to increased reactive oxygen species. There is a significant increased in protein glycation (AGE) with age, which is also increased in diabetics (Khalid, 2007).

Aging is the wear and tear on human organism associated with each day of living, this wear and tear is largely caused by free radical reactive species. Sundaram *et al.* (2005) studied patient with NIDDM in their fifth and sixth decades and showed an increase in lipid peroxides from the on set of disease. This became progressively worse with time and with development of complications. The increased oxidative damage that was demonstrated in elderly patients with NIDDM may therefore predispose to the development of atherosclerosis. This is apparent despite comparable antioxidant defense in age – matched control (Giualiano *et al.,* 1995). In hypertensive patients the age group

between 20-35years was compared with 36-55 years, elevated level of glucose, lipid peroxides, total cholesterol and LDL cholesterol were observed. Also decrease total antioxidant and HDL cholesterol were observed in 36-55 as compared with 20-35 years. Oxidative stress has been implicated in many diseases associated with ageing (Ames *et al.*, 2003) and in the ageing process itself (Pryor, 2007).

Recent report shows that advanced age, in the absence of disease, is associated with decreased antioxidant defense and increased oxidative damage (Nuhill *et al.*,1998). Elderly diabetes-hypertensive patients are more susceptible to free – radical – induced damage that can be accounted for by normal ageing (Corella *et al.*, 2001). Oxidation of low-density lipoprotein (LDL) and total cholesterol is a key step in the formation of atheroma and is thought to be a major factor in the development of cardiovascular diseases (Lones *et al.*, 1985). This may be of particular importance in patients with NIDDM, because in addition to raised total LDL cholesterol level, they tend to have a pro – artherogenic lipid profile composed of small dense LDL particles and raised total cholesterol (Nuhill *et al.*, 1998).

The reduction in total antioxidant status was associated with increasing HbA1c and duration of diabetes (Jonathan 2007; Avil, 2007). A reduction in total antioxidant and increased in lipid peroxides and lipid profiles is also associated with acceleration of the natural age–dependent, increases in oxidative stress, oxidative damage and may cause diabetes-hypertension (Ligia *et al*., 2010). Further finding stated that, in African communities, the prevalence is increasing with ageing of the population and life style changes associated with urbanization (Sobngwi *et al.*, 2001). The prevalence of diabetes in suburban population of Northern Nigeria is 1.6% **(**Bakari *et al.*, 1999**).**

Diabetes is a free radical associated disease. Investigations carried out in diabetic patients revealed oxidative stress load (Packer, 2002). Oxidative destruction of subcellular membrane lipids has been implicated along with other types of intracellular oxidative damage in the normal aging process and in pathophysiology of a number of chronic illnesses. Complex antioxidant mechanism, including antioxidant vitamins, exists to limit the effects of these reactions (Packer *et al.*, 2002).

In view of the significantly reduced antioxidant concentrations in diabetic subjects obtained in this study, and significant negative correlations between serum antioxidant and HDL with increased serum glucose, lipid peroxide, total cholesterol, triglyceride and LDL level of these subjects was as a result of diabetic complication (Muhammed *et al.*, 2005). The development of diabetic late complications (cataract, retinopathy, nephropathy and neuropathy) is associated with an increased presence of free radicals, and, therefore elevated oxidative stress of the human body (packer *et al.*, 2000). Hyperglycemia is a recognized pathogenic factor of long-term complications in diabetes mellitus. It does not only generate reactive oxygen species but also attenuates antioxidant mechanism, creating a state of oxidative stress (Catherwood *et al.*, 2002). The beta cells are sensitive to oxidative stress because their intracellular antioxidant defense mechanisms are weak (Cariello *et al.*, 1992). It is believed that one of the mechanism responsible for secondary complications of diabetes involve non-enzymatic glycosylation of proteins by glucose auto-oxidation (Cariello *et al.*, 1992). Non enzymatic glycation is a spontaneous chemical reaction between glucose and the amino groups of proteins resulting in the formation of reversible Schiff's bases and Amadori products. These products have been reported to generate free radicals, causing oxidative stress and tissue damage (Baynes, 1991).

Antioxidant vitamins such as vitamin C and E have been shown to reduce protein glycosylation and act as scavengers of free radicals generated by glycosylated proteins (Cariello *et al.*, 1992). Cardiovascular complications of diabetes are, in part due to small vessel damage by oxidized low-density lipoprotein (LDL). Vitamin E was found to lower LDL oxidation, thus lowering the risk of diabetic cardiovascular complications (Fuller *et al.*, 1996). Negative correlation between glucose and total antioxidant among diabetics-hypertensive subjects was observed.

The decrease in total antioxidant and HDL, with increased in lipid peroxide, cholesterol and LDL confirm with epidemiologic studies the evidence for co-existence of hypertension and diabetes and possibly point towards a common genetic and environmental factors promoting both diabetes and hypertension. Similarly, clustering of hypertension, insulin resistance or type 2 diabetes, hyperlipidaemia and central obesity

have been documented in several populations. Insulin resistance, increased tissue inflammation and reactive oxygen species (ROS) production resulting in endothelial dysfunction, increased tissue renin– angiotensin–aldosterone system (RAAS) and increased sympathetic nervous system (SNS) activity have all been implicated in this complex pathophysiology of diabetes and hypertension. (Sowers and Haffner, 2002). The study indicated a weak, yet positive significant association between elevated blood glucose concentrations and low concentrations of HDL-C. Hyperglycemia progressively increases the transfer of cholesterol esters from HDL-C to VLDL-C particles (Goldberg, 2001). The denser LDL particles acquire a large proportion of these HDL esters, further diminishing the HDL-C levels. In addition, HDL-C is a ready substrate for hepatic lipase which converts it into smaller particles that are readily cleared from the plasma (Horowitz *el al*., 1993). The relative insulin deficiency that occurs in type 2 diabetes impairs the action of lipoprotein lipase and results in lower HDL-C levels and higher triglyceride levels, which may improve with improved glycemic control (Brunzell and Chait 1997). Thus, HDL hypocholesterolemia in type 2 diabetes patients is mainly due to insulin resistance-linked lipoprotein lipase deficiency and a reduction in HDL_2 sub-fraction which is secondary to increased HDL-C catabolism (Verges, 1999).

Negative correlation was found between total antioxidant and lipid profile among diabetes subjects and diabetic-hypertensive subjects with decrease in HDL. This work confirmed the work of Gordon *et al.* (2010), that HDL acts by enhancing the removal of cholesterol from the peripheral tissues and so reduces the body's cholesterol pool. Type 2 DM was usually associated with low plasma levels of HDL-C (Barrette and Wingard, 1983).

CHAPTER SIX

CONCLUSION, RECOMMENDATION AND CONTRIBUTION TO KNOWLEDGE

Conclusion

There is a decrease in the total antioxidant level and increase lipid peroxidation in diabetic, hypertensive and diabetic-hypertensive patients. Increase total cholesterol and low density lipoprotein with decrease in high density lipoprotein were also associated with higher incidence of diabetic and hypertensive complications.

In elderly people the level of glucose is higher in diabetic patients than younger age. Decrease in total antioxidant and high density liprotein levels with increase in lipid peroxidation, total cholesterol and low density lipoprotein is accompanied with progress in aging.

The result shows negative correlation between total antioxidant and high density lipoprotein with increase in glucose level. While positive correlation was found between lipid peroxide, total cholesterol and low density lipoprotein with increase in glucose level for both diabetic, hypertensive and diabetic-hypertensive patients.

It is conclude that age-related decline in plasma increase lipid peroxidation and lipid profiles with decrease in total antioxidant play an important role in age-related accumulation of cell damage caused by reactive oxygen species.

6.2 Recommendation

Based on this work it is recommended that total antioxidant and lipid peroxidation assay be introduced as part of routine check up for those that have diabetic and hypertension.

6.3 Contribution to knowledge

- As a result of this work, it was discovered that diabetic, hypertensive and diabetic-hypertensive patients were accompanied with decrease in total antioxidant status level with increase in lipid peroxidation and lipid profile levels.

- It was also shown that people with complications of diabetes-hypertension suffer more of the changes in the biochemical parameters than those that have diabetes or hypertension alone.
- There is a decrease in total antioxidant level with an increase in lipid peroxidation and lipid profile levels of elderly patients than younger patients'
- This work shows positive association between high glucose level and total antioxidants with negative correlation between lipid peroxidation and lipid profile of diabetic, hypertensive and diabetic-hypertensive patients.

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