OXIDATIVE STRESS AND DYSLIPIDAEMIA IN TYPE -2 DIABETIC PATIENTS ATTENDING CHUKWUEMEKA ODUMEGWU OJUKWU UNIVERSITY TEACHING HOSPITAL, AWKA.

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CERTIFICATION

We hereby certify that this thesis "**Oxidative Stress and Dyslipidaemia In Type -2 Diabetic Patients Attending Chukwuemeka Odumegwu Ojukwu University Teaching Hospital Awka**." Was carried out by **NWANKWO CHINENYE ESTHER (20144912678)** and accepted in partial fulfillment for the award of Masters (M.Sc.) degree in Medical Biochemistry in the Department of Biochemistry, Federal University of Technology, Owerri.

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DEDICATION

To God, who continues to uphold me

&

My Parents of blessed memory

(Daniel & Agnes)

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ABSTRACT

Changes in oxidative stress parameters, lipid profile, atherogenic predictor indices, and anthropometrics of type 2 diabetes mellitus (T2DM) subjects attending Chukwuemeka Odumegwu Ojukwu University Teaching Hospital (COOUTH) Awka, were assessed using standard methods. Demographic data of the T2DM patients were collected with the aid of structured questionnaire.The subjects included willingly consented 60 (30 males and 30 females) T2DM patients and 40 (20 males and 20 females) apparently healthy, age-matched control subjects.GSH concentration, and activities of SOD, CAT and GPx decreased significantly ($p<0.05$), while MDA level increased significantly ($p<0.05$) in diabetics than in non-diabetics. The FBG concentration, lipid parameters (TC, TG, LDL-c and VLDL-c) and atherogenic indices (non HDL-c, CRI 1, CRI 11, AC and AIP) were significantly ($p<0.05$) higher in the diabetics, while HDL-c concentration was significantly decreased in diabetics compared to the controls. Majority of the diabetic subjects had abnormally high prevalence of dyslipidaemia and abnormally high levels of atherogenic predictor indices. The diabetics had significantly ($p<0.05$) higher BMI, WC, AC, WHR, WHtR, SBP and DBP than non-diabetics. FBG results of diabetics correlated positively with MDA ($r^2= 0.30$, $p=0.02$) and non HDL-c ($r=0.28$; $p=0.027$), but negatively with GSH ($r^2= -0.30$, $p=0.04$). Arm circumference had a weak negative correlation with GSH and CAT in the diabetics. BMI, WHtR, SBP and DBP were positively correlated with non HDL-c, CRI-I, CRI-II and AC, while AIP was positively correlated with BMI and WHtR. Diabetic males compared to females, were more educated, resident in urban areas, had higher co-morbidity with hypertension, showed more signs of complications, had family history of diabetes and had over 10 years of suffering fromT2DM. On the other hand, female diabetics were involved in lower income occupation and practiced better nutritional regimen, but had significantly ($p < 0.05$) higher BMI, WC, WHR, SBP and DBP than the males. The results of this study have shown high occurrence of dyslipidaemia, oxidative stress and abnormality in atherogenic risk predictor indices among Nigerian T2DM patients. Correlation results indicate that MDA, non HDL-c and GSH are potentially important useful markers for the diagnosis and/or prognosis of diabetes. Adoption of a healthy dietary pattern should be encouraged in T2DM patients and intervention policies for effective management of T2DM should be sex specific.

Keywords: Hyperglycemia, lipid profile, ROS, anthropometry, atherogenic index, Awka.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Diabetes is any disorder characterized by excessive urine excretion, which includes **Diabetes mellitus** (the most common form and the type studied in this research work), **diabetes insipidus** and **brittle diabetes**. Diabetes insipidus is the result of a deficiency of antidiuretic hormone(ADH, also referred to as vasopressin or arginine vasopressin, AVP). The major symptom of diabetes insipidus (excessive output of dilute urine) results from an inability of the kidneys to reabsorb water. Brittle diabetes is a form that is very difficult to control. It is characterized by unexplained oscillations between hypoglycaemia and acidosis (Michael, 2016).Diabetes mellitus is derived from the Greek word "diabetes" meaning siphon - to pass through and the Latin word "mellitus" meaning honeyed or sweet.This is because in diabetes mellitus, excess sugar (glucose) is found in blood as well as in the urine. It was known in the 17th century as the "pissing evil" (Mandal, 2012).

Diabetes mellitus (DM) is an aetiologically multifactorial metabolic disorder, characterized by chronic hyperglycaemia, which arise due to defects in insulin secretion, and/or action. Insulin is a hormone manufactured by the beta cells of the pancreas, which is required to utilize glucose from digested food as an energy source. Insulin defects result in aberrations in carbohydrate, fat and protein metabolism (Bos & Agyemang, 2013). Although the aetiology of this disease is not well defined, viral infection, autoimmune disease, and environmental factors have been implicated (Sander, Anderson, &Barbu, 2000). The basic effect of lack of insulin or insulin resistance on glucose metabolism, in the disease condition of diabetes mellitus, is to prevent the efficient uptake and utilization of glucose by most cells of the body, except those of the brain. As a result of this, blood glucose concentration increases, cell utilization of glucose falls increasingly lower and utilization of fats and proteins increases. (Guyton &Hall, 2006a). There are two main types of diabetes mellitus viz : Type 1 diabetes, previously known as the insulin dependent diabetes mellitus (IDDM), which is caused by lack of insulin secretion by beta cells of the pancreas, and Type 2 diabetes, previously known as non-insulin dependent diabetes mellitus (NIDDM), which is caused by decreased sensitivity of target tissues to insulin (Ozougwu,Obimba, Belonwu, & Unakalamba, 2013).Type 2 DM is the commonlydocumented

form of DMand in mostendocrine clinics, it accounts for about90%-95% of all cases of DM (Ogbera &Ekpebegh, 2014). Patients with type 2 diabetes suffer from several complications such as atherosclerosis, retinopathy, neuropathy and nephropathy with devastating effect on morbidity and mortality (Dandona, Thusu & Cook, 1996).All these complications have long-lasting adverse effects on a nation's health and economy, especially for developing countries.

Diabetes is a chronic metabolic disease with high morbidity, mortality and prevalence (Ogbonna,Opara, Ezenduka & Udochukwu, 2013). In 2015, 415 million people have diabetes in the world, and by 2040 this figure will more thandouble. There were more than 1.56 million cases ofdiabetes in Nigeria in 2015(IDF, 2015a). Data from the World Health Organization (WHO) suggests that Nigeria has the greatest number of people living with diabetes in Africa (Wild, Roglic, Green, Sicree, & King, 2004). Diabetes is poised to affect the developing countries of the world much more than their developed counterparts (Kengne,Amoah, & Mbanya, 2005). Escalation of the incidence of diabetes in developing countries (like Nigeria) follows the trend of urbanization and lifestyle changes, perhaps most importantly a "western-style" diet.

'Oxidative stress', defined as an imbalance between oxidants and cellular antioxidant defence system, has been implicated strongly in favour of the pathogenesis of diabetes mellitus and its complications (Baynes,1991;Giugliano, Ceriello & Paolisso, 1996). Hyperglycaemia generates reactive oxygen species (ROS), which in turn cause damage to the cells in many ways. Damage to the cells ultimately results in secondary complications in diabetes mellitus (Jaganjac, Tirosh, Cohen, Sasson, & Zarkovic, 2013).

Dyslipidaemia is an abnormal amount of lipids (e.g. cholesterol and/or fat) in the blood. It is very common among people with Type 2 diabetes. Dyslipidaemia is one of the major risk factors for cardiovascular disease in diabetes mellitus. The lipid changes associated with diabetes mellitus are attributed to increased free fatty acid flux secondary to insulin resistance (Mooradian, 2009; ADA (American Diabetes Association), 2015).

Dyslipidemia and oxidative stress are associated with DM and these represent a serious public health problem in our society. This study was designed to determine the lipid profile and the intensity of oxidative stress among type 2 diabetic subjects in comparison with non-diabetic subjects. Also the risk of diabetes complications was assessed through anthropometric and atherogenic indices of the patients.

1.2 Justification for the study

The prevalence of diabetes is rising all over the world due to population growth, aging, diet, and the increase of obesity due to physical inactivity. Diabetes and its complications bring about substantial economic loss to the diabetics and their families, as well as the health systems and national economies through direct medical costs and loss of jobs (WHO, 2016). About 12% of global health expenditure is spent on diabetes (IDF, 2015b).Almost 80% of diabetes deaths occur in low and middle-income countries (WHO, 2010), which the population under study is a typical example. Moreover, no such research on diabetes has been carried out in the hospital used as the case study in this research. Furthermore, the need to continue to explore the relationship between oxidative stress, dyslipidaemia and their roles in diabetes mellitus, and its complications in an effort to better understand the disease and expand treatment options cannot be overemphasized. We propose that type 2 diabetics who are at increased risk of developing diabetes complications could also be identified by analyzing anthropometric and atherogenic indices. Data obtained from the study will assist the clinicians in management of type 2 DM.

1.3 Aim and objectives

The aim of this study was to investigate oxidative stress and dyslipidaemia and their relationships with anthropometric and atherogenic indices of type -2 diabetic patients attending Chukwuemaka Odumegwu Ojukwu University Teaching Hospital, Awka, compared to the apparently healthy individuals with the following objectives.

1.To determine the levels of oxidative stress markers, (including glutathione (GSH), superoxide dismutase(SOD), catalase (CAT), glutathione peroxidase (GPx), and malondialdehyde (MDA)in type 2 diabetic patients.

2. To determine the lipid profile (total cholesterol, triglycerides (TG), high density lipoproteins (HDL), low density lipoproteins (LDL), and very low density lipoproteins (VLDL) of the diabetic patients.

3. To determine the levels of oxidative stress and lipid profile in apparently healthy individuals.

4. To estimate the blood glucose level in both patients and controls.

5. To measure and correlate the anthropometric indices -Waist Circumference (WC), Body Mass Index (BMI), Waist to Hip Ratio (WHR), Arm circumference (AC) and waist- to- height ratio (WHtR) of the diabetic subjects with their biochemical parameters.

6. To calculate and correlate the atherogenic indices- non high density lipoprotein cholesterol (nHDL-c),castelli's Risk Index1 (CRI-I) and 2 (CRI-II), atherogenic coefficient (AC) and atherogenic index of plasma (AIP) of the diabetic subjects with other biochemical parameters.

CHAPTER TWO

LITERATURE REVIEW

2.1 Brief history of diabetes mellitus

The earliest known record of diabetes was written on a 3rd Dynasty Egyptian papyrus in **1552** BC in which the physician Hesy-Ra mentioned frequent urination as a symptom. Ancient Hindu writings noted in 1500 BC that ants are attracted to the urine of people with a mysterious emaciating disease. The first description of sugar in the urine and its occurrence in obese individuals was in 500 BC (Swidorski, 2014).

The term diabetes was coined by Apollonius of Memphis around 250 BC meaning to go through, or siphon, for a disease that drains patients of more fluid than they can consume. It was in 1675 that Thomas Willis added the word "'mellitus'" meaning honeyed or sweet, to the word diabetes. This was because of the sweet taste of the urine. This sweet taste had been noticed in urine by the ancient Greeks, Chinese, Egyptians, Indians, and Persians as is evident from their literature (Mandal, 2012).

2.2 Types of diabetes mellitus

Terms that describe the age of onset (juvenile or adult) or type of treatment (insulin - or non–insulin -dependent) are no longer accurate in diabetes classification because of overlap in age groups and treatments between disease types (Preeti, 2014)**.** Presently, the classification of diabetes is based on the presumed aetiology(Loghmani, 2012), and combination of other features (Preeti, 2014)**.**

There are three main types of diabetes mellitus, types 1 and 2 and gestational diabetes(IDF, 2015b). The 2 most common categories are—type 1 and type 2. Type 1 and type 2 diabeteswere identified as separate conditions for the first time by the Indian physicians Sushruta and Charaka,with type 1 associated with youth and type 2 with obesity (Poretsky,2009). In type 1 diabetes, the body does not produce insulin, and daily insulin injections are required, usually diagnosed during childhood or early adolescence. Type 2 diabetes is the result of failure to produce sufficient insulin and insulin resistance. It is typically diagnosed during adulthood (Loghmani, 2012). However with the increasing incidence of childhood obesity and concurrent insulin resistance, the number of children diagnosed with type 2 diabetes has also increased worldwide

(Rosenbloom, Joe, Young, & Winter, 1999).The following aetiology/contributing factors, as well as clinical characteristics clearly distinguish between the two types of diabetes mellitus.

2.2.1 Type 1 diabetes

Type 1 diabetes is caused by the immune destruction of the beta cells of the pancreas.Antibodies to islet cells and insulin are present at diagnosis, and then gradually insulin secretion diminishes(Loghmani, 2012)Type 1 diabetes may occur at any age, but most common in childhood and adolescence. Insulin injection is necessary for survival. Genetic predisposition and environmental factors (infection or other stress) are the contributing factors.

2.2.2 Type 2 diabetes

Type 2 diabetesis caused by insulin resistance in the liver and skeletal muscle, increased glucose production in the liver, over production of free fatty acids by fat cells and relative insulin deficiency(Loghmani, 2012).Insulin secretion decreases with gradual beta cell failure. Reductions in blood glucose levels often can be achieved with changes in food intake and physical activity patterns, however,oral medication and/or insulin injections are eventually required.Obesity, age (onset of puberty is associated with increased insulin resistance),lack of physical activity, genetic predisposition, racial/ethnic background (African American, nativeAmerican, Hispanic and Asian/Pacific Islander) and conditions associated with insulin resistance, (e.g., polycystic ovary syndrome) are the contributing factors (Loghmani, 2012).

2.2.3 Gestational diabetes

Gestational diabetes occurs when pregnant women without a previous history of diabetes develop high blood-sugar levels. Pregnancy causes some insulin resistance in all women, but only a few develop gestational DM (Preeti, 2014)**.**Gestational diabetes can lead to serious health risks for both the mother and the child, and it is associated with an increased risk of both mother and child developing type 2 diabetes later in life (IDF, 2015b).

2.3 Symptoms and signs of diabetes mellitus

The most common symptoms of DM are those of hyperglycaemia. The mild hyperglycaemia of early DM is often asymptomatic; therefore, diagnosis may be delayed for many years. More significant hyperglycaemia causes glycosuria and thus an osmotic diuresis, leading to increase in urinary frequency, polyuria, and polydipsia that may progress to orthostatic hypotension and dehydration (Preeti, 2014)**.** Severe dehydration causes weakness, fatigue, and changes in mental

status. Symptoms may come and go as plasma glucose levels fluctuate. Polyphagia may accompany symptoms of hyperglycaemia, but is not typically a patient's primary concern. Hyperglycaemia can also cause weight loss, nausea and vomiting, and blurred vision, and it may predispose one to bacterial or fungal infections (Preeti, 2014)**.**

Patients with type 1 DMtypically present with symptomatic hyperglycaemia and sometimes with diabetic ketoacidosis (DKA). Some patients experience a long but transient phase of near-normal glucose levels after acute onset of the disease (honeymoon phase) due to partial recovery of insulin secretion.

Patients with type 2 DMmay present with symptomatic hyperglycaemia but are often asymptomatic, and their condition is detected only during routine testing. In some patients, initial symptoms are those of diabetic complications, suggesting that the disease has been present for some time. In some patients, hyperosmotic coma occurs initially, especially during a period of stress or when glucose metabolism is further impaired by drugs, such as corticosteroids (Preeti, 2014)**.**

2.4 Epidemiology and mortality of type 2 diabetes mellitus

Type 2 diabetes is the most prevalent form of diabetes and has increased alongside cultural and societal changes (IDF, 2015b). Globally as at 2010, it was estimated that there were 285 million people with type 2 diabetes making up about 90% of diabetes cases (Melmed, Polonsky, ReedLarson, & Kronenberg, 2011). It is estimated that 439 million people would have type 2 DM by the year 2030 (Chamnan, Simmons, Forouhi, Luben, Khaw and Wareham, 2011). In 2015, 415 million adults (20-79 years) were estimated to have diabetes worldwide, and by 2040 this will be 642 million (IDF, 2015b). Since type 2diabetes is associated with older age and is more prevalent than type 1, about 90% of this 642 million people will likely be type 2 diabetics. An estimated 14.2 million adults aged 20-79 have diabetes in the African region in 2015, and will rise to 34.2 million in 2040 (IDF, 2015b). About 1.9% of Nigerians were diabetic in 2015(IDF, 2015a). The incidence of type 2 DM varies substantially from one geographical region to the other as a result of environmental and lifestyle risk factors (Zimmet,Alberti, & Shaw, 2001).

Type 2 diabetes is a heterogenous disorder caused by a combination of genetic factors related to impaired insulin secretion, insulin resistance and environmental factors such as obesity, over

eating, lack of exercise, stress as well as aging (Kaku, 2010). It is typically a multifactorial disease involving multiple genes and environmental factors to varying extents (Holt, 2004; Winter, 2014). It is estimated by IDF that 193 million people with diabetes worldwide and more than two thirds of diabetics in Africaare undiagnosed and are therefore more at risk of developing complications. Furthermore, one in 15 adults is estimated to have impaired glucose tolerance, and one in seven births is affected by gestational diabetes (IDF, 2015a & b). Both of these conditions are associated with an increased risk of developing type 2 diabetes in later life.
Poorly managed diabetes leads to serious complications and early death. Diabetes and its complications are major causes of death in most countries. Adults who died from diabetes in 2015 were 5.0 million (IDF, 2015b). Greater education is needed to embed lifestyle changes (such as proper diet and exercise) that will reduce the rise in type 2 diabetes.

2.5 Pathogenesis of type 2 diabetes

Type 2 diabetes arises when insulin resistance-induced compensatory insulin secretion is exhausted (Michael, 2016). Under normal physiological conditions, plasma glucose concentrations are maintained within a narrow range, despite wide fluctuations in supply and demand, through a tightly regulated and dynamic interaction between tissue sensitivity to insulin (especially in liver) and insulin secretion (DeFronzo & Ferrannini, 1988). In type 2 diabetes, these mechanisms break down, hence the two main pathological defects in type 2 diabetes are impaired insulin secretion through a dysfunction of the pancreatic β-cell, and impaired insulin action through insulin resistance (Holt, 2004)(Figure2. 1)

Insulin resistance by itself may be a secondary event in type 2 DM, since it is also found in non-diabetic obese individuals. Insulin secretion defect may be the primary event, presenting as impaired pulsatile secretion of insulin. Hence, hyperglycaemia is an inducer (high glucose levels desensitize β cells, cause β-cell dysfunction (glucose toxicity) or both)(Preeti Kishore, 2014), as well as a consequence of impaired islet cell function and insulin resistance (Ozougwu *et al,* 2013).

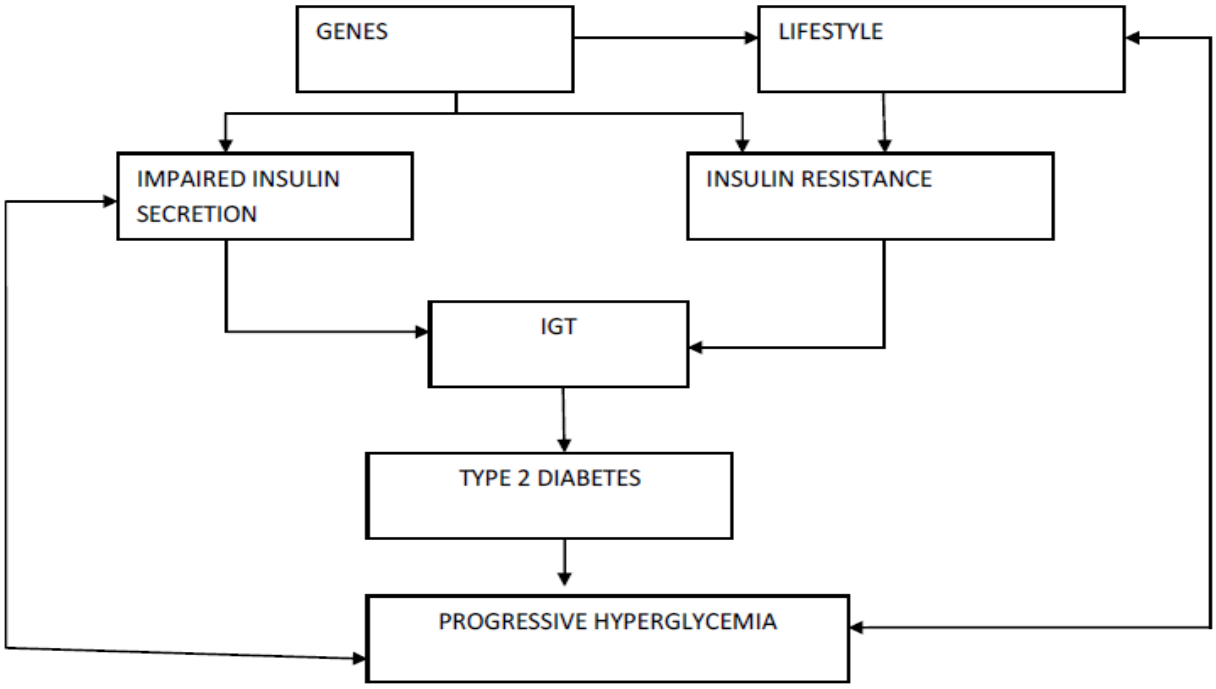

Figure 2.1: Pathogenesis of type 2 diabetes characterized by impaired insulin secretion and insulin resistance. (Ozougwu *et al.*, 2013). (IGT, impaired glucose tolerance)

The development of type 2 diabetes is caused by a combination of lifestyle (age, lack of exercise, increased dietary fat, decreased fibres) and genetic factors (Ozougwu *et al.*, 2013), whichcontribute to the insulin insensitivity, including obesity and its duration (Evephart,Pettit, Bennett, & Knowler, 1992).

There is a strong inheritable genetic connection in type 2 DM. Having relatives (especially first degree) with type 2 DM increases the risks of developing type 2 DM substantially. Concordance among monozygotic twins is close to 100%, and about 25% of those with the disease have a family history of DM (Rother, 2007). In general, mutations in any gene involved in glucose regulation can affect the risk of type 2 diabetes. These include genes that control production of glucose and/or insulin, regulation of insulin and glucose sensitization level in the body (Winter, 2014). Mutations in the insulin gene lead to the synthesis and secretion of abnormal gene products, leading to insulinopathies (Gabbay, 1980). Most of the patients with insulinopathies have hyperinsulinaemia, inherited in autosomal fashion, heterozygous for normal and mutant alleles, and normally respond to exogenous insulin administration. Most insulin gene mutations lead to:

(a) Abnormal insulins - where the mutation leads to an amino acid replacement at an important site for receptor interaction; or

(b) The mutation may interfere in the proinsulin processing to insulin (Al Homsi & Lukic, 1992; Chan, Malik, Jia, Kadowaki, Yajnik, Yoon, & Hu, 2009).

Genes discovered to be significantly associated with the development of type 2 DM, include *TCF7L2*, *PPARG*, *FTO*, *KCNJ11*, *NOTCH2*, *WFS1*, *CDKAL1*, *IGF2BP2*, *SLC30A8*, *JAZF1*, and *HHEX*. *KCNJ11* (potassium inwardly rectifying channel, subfamily J, member 11), which encodes the islet ATP-sensitive potassium channel Kir6.2, and *TCF7L2* (transcription factor 7-like 2) which regulates proglucagon gene expression and thus the production of glucagon-like peptide-1(McCarthy, 2010). Moreover, obesity (which is an independent risk factor for type 2 DM) is strongly inherited (Walley, Blakemore, & Froguel, 2006). Monogenic forms like maturity-onset diabetes of the young (MODY), constitutes up to 5% of type two diabetes cases (Camastra,Bonora, Del Prato, Rett, Weck & Ferrannini, 1999).Mutations in 10-12 different genes have been correlated with the development of MODY (Michael, 2016). There are a number of medications (e.g. glucocorticoids, thiazides, beta blockers, atypical antipsychotics(Izzedine,Launay-Vacher, Deybach, Bourry, Barrou, & Deray, 2005), statins(Sampson, Linton, & Fazio, 2011),and other health problems (e.g. acromegaly, Cushing's syndrome, hyperthyroidism, pheochromocytoma, and certain cancers such as glucagonomas, (Feinglos & Angelyn, 2008)that can predispose to diabetes. Consumption of sugar-sweetened drinks in excess is associated with an increased risk(Malik, Popkin, Bray, Després, Willett,& Hu2010). The type of fats in the diet are also important, with saturated fats and trans fatty acids increasing the risk, whilepolyunsaturated and monounsaturated fat decrease the risk (Risérus,Willett&Hu,2009).Eating much of white rice appears to also play a role in increasing risk(Hu,Pan, Malik, & Sun, 2012).

2.6 Pathophysiology of type 2 diabetes

Type 2 DM is characterized by insulin insensitivity as a result of insulin resistance, declining insulin production, and eventual pancreatic beta-cell failure (Kahn &Banting, 1994). This leads to a decrease in glucose transport into the liver, muscle cells, and fat cells, increased breakdown

of lipids within fat cells, resistance to and lack of incretin, high glucagon levels in the blood, and inappropriate regulation of metabolism by the central nervous system (Melmed, Polonsky, Reed Larsen, &Kronenberg,2011). However, not all people with insulin resistance develop diabetes, since an impairment of insulin secretion by pancreatic beta cells is also required. The involvement of impaired alpha-cell function has been recognized in the pathophysiology of type 2 DM (Fujioka, 2007).

Unlike patients with type 1diabetes, those with type 2diabetes have detectable levels of circulating insulin. On the basis of oral glucose tolerance test, the essential elements of type 2 diabetes can be divided into 4 distinct groups; those with normal glucose tolerance, chemical diabetes (called impaired glucose tolerance), diabetes with minimal fasting hyperglycaemia (fasting plasma glucose <140 mg/dL), and diabetes mellitus in association with overt fasting hyperglycaemia (fasting plasma glucose >140 mg/dL) (Michael, 2016). In patients with the highest levels of plasma insulin (impaired glucose tolerance group) there is also elevated plasma glucose. This indicates that these individuals are resistant to the action of insulin. In the progression from impaired glucose tolerance to diabetes mellitus the level of insulin declines indicating that patients with type 2 diabetes have decreased insulin secretion (Michael, 2016).

A majority of individuals suffering from type 2 DM are obese, with central visceral adiposity. The predominant theory used to explain this link is the portal/visceral hypothesis giving a key role in elevated non-esterified fatty acid concentrations (Fujioka, 2007). Also in hyperglycaemia, the level of glucose excretion is increased by the kidneys leading to frequent urination (polyuria). As the glucose is excreted there is a concomitant loss of water to maintain normal osmolarity of the urine. The water loss thus consequently leads to excessive thirst called polydypsia.

2.7 Diabetic complications

Years of poorly controlled hyperglycaemia lead to multiple, primarily vascular complications that affect small vessels (microvascular), large vessels (macrovascular), or both. The mechanisms by which vascular disease develops include glycosylation of serum and tissue proteins with formation of advanced glycation end products; superoxide production; activation of protein kinase C, a signaling molecule that increases vascular permeability and causes endothelial dysfunction; accelerated hexosamine biosynthetic and polyol pathways leading to sorbitol accumulation within tissues. Other mechanisms are hypertension and dyslipidaemias that

commonly accompany DM; arterial microthromboses; and proinflammatory and prothrombotic effects of hyperglycaemia and hyperinsulinaemia that impair vascular autoregulation. Immune dysfunction is another major complication and develops from the direct effects of hyperglycaemia on cellular immunity (Preeti, 2014). Persistent and increasing hyperglycaemia also makes the blood more viscous which makes circulation of the blood in the small capillaries difficult. The reduced circulation results in progressive vascular complications leading to diabetic retinopathy (referred to as diabetic blindness), peripheral neuropathy (resulting in numbness in the extremities and tingling in fingers and toes), poor wound healing, and erectile dysfunction (Michael, 2016). Diabetic nephropathy (glomerular sclerosis and fibrosis) can result from protein glycosylation and haemodynamic changes of diabetes mellitus. It manifests as slowly progressive albuminuria with worsening hypertension and renal insufficiency.Microvascular diseases (retinopathy, neuropathy and nephropathy) underlie the 3 most common and devastating manifestations of DM. Macrovascular disease (large-vessel atherosclerosis) is a result of the hyperinsulinaemia, dyslipidaemias, and hyperglycaemia characteristic of DM. Manifestations are angina pectoris and myocardial infarction (MI), transient ischemic attacks and strokes, and peripheral arterial disease.

Other complications include cardiomyopathy and infection. Diabetic cardiomyopathy is thought to result from many factors, including epicardial atherosclerosis, hypertension and left ventricular hypertrophy, microvascular disease, endothelial and autonomic dysfunction, obesity, and metabolic disturbances. Patients develop heart failure due to impairment in left ventricular systolic and diastolic function and are more likely to develop heart failure after MI. Patients with poorly controlled DM are prone to bacterial and fungal infections because of adverse effects of hyperglycaemia on granulocyte and T-cell function. Most common are mucocutaneous fungal infections (e.g., oral and vaginal candidiasis) and bacterial foot infections (including osteomyelitis), which are typically exacerbated by lower extremity vascular insufficiency and diabetic neuropathy (Preeti, 2014). Intensive control of plasma glucose can prevent or delay many of these complications but may not reverse them once established (Helena, 2016).

2.8 Diagnosis and screening of type 2 diabetes mellitus

Table 2.1: Diagnostic Criteria for Diabetes Mellitus and Impaired Glucose Regulation.

Test	Normal	Impaired Glucose Regulation	Diabetes
FPG (mg/dl[mmol/L])	<100(<5.6)	100-125(5.6-6.9)	≥126 (≥7.0)
OGTT (mg/dl[mmol/L])	<140(<7.7)	140-199(7.7-11.0)	≥200 (≥ 11.1)
HbA_{1c} (%)	<5.7	5.7-6.4	≥ 6.5

FPG = fasting plasma glucose; HbA_{1c} = glycosylated Hb; OGTT = oral glucose tolerance test, 2-h glucose level (Preeti, 2014).

According to World Health Organization National Diabetic Group Criteria of 2006 and the American Diabetic Association (ADA) guidelines of 1997, definition of diabetes (both type 1 and type 2) is for a single raised glucose reading with symptoms (polyuria, polydipsia, polyphagia and weight loss). Raised values on two occasions, of either FPG ≥ 7.0 mmol/l (126 mg/dl) or OGTT (two hours after the oral dose of 75g of glucose) ≥ 11.1 mmol/l (200 mg/dl) also defines diabetes (Cox & Elelman, 2009; IDF, 2015b). A random blood sugar of greater than 11.1 mmol/l (200 mg/dL) in association with typical symptoms is another method of diagnosing diabetes(Vijan, 2010).Glycated haemoglobin (HbA_{1c}) of ≥ 0.6%, was also adopted by the American Diabetes Association in 2010 (American Diabetes Association, 2010).Positive tests should be repeated unless the person presents with typical symptoms and blood sugars >11.1 mmol/l (>200 mg/dl]) (International Expert Committee, 2009).

2.8.1 Impaired glucose regulation:

People with raised blood glucose levels or percentage of HbA_{1c} that are not high enough for a diagnosis of diabetes are said to have impaired glucose regulation (i.e. impaired glucose tolerance (IGT) or impaired fasting glucose (IFG) in the case of glucose levels. These conditions are sometimes called “pre-diabetes” or at risk of DM(Table 2.1) (Preeti, 2014; IDF, 2015b).Thus the test recommended for screening is the same as that for making diagnosis, with the result that a positive screen is equivalent to a diagnosis of pre-diabetes or DM. (Cox

&Elelman, 2009). People with impaired glucose tolerance are at increased risk of developing type 2 diabetes. Impaired glucose tolerance shares many characteristics with type 2 diabetes and is associated with advancing age and the inability of the body to use the insulin it produces. Not everyone with impaired glucose tolerance goes on to develop type 2 diabetes; a large body of evidence supports the effectiveness of lifestyle interventions – healthy diet and physical exercise – in preventing the progression to diabetes (IDF, 2015b).

Type 1 and type 2 diabetes can be distinguished during diagnosis based on the presenting signs and symptoms. For instance, symptom onset is gradual for type 2 and rapid for type 1; ketoacidosis is always present in type1 at the time of diagnosis but not in type 2; weight loss in type 1, but obese with increased abdominal girth in type 2.If diagnosis is in doubt, antibody testing may be useful to confirm type 1 diabetes, and C-peptide levels may be useful to confirm type 2 diabetes *(*Lippincott & Wilkins, 2007),with C-peptide levels normal or high in type 2 diabetes, but low in type 1 diabetes(Mary, 2013).

2.9 Treatment of type 2 diabetes

A major goal of therapeutic intervention in type 2 diabetes is to control hyperglycaemia to relieve symptoms and prevent complications while minimizing hypoglycaemic episodes (Michael, 2016; Preeti, 2014; Loghmani, 2012). The first measures to control type 2 diabetes blood glucose are often meal planning (eating high fibre and unsaturated fat and diet low in saturated and trans-fats and glycemic index), weight loss measures, exercising, maintenance of body mass index of 25 kg/m^2, abstinence from smoking and moderate consumption of alcohol(Olokoba, Obateru, &Olokoba,2012; Preeti, 2014; Loghmani, 2012). Sometimes these measures are not enough to reduce blood glucose levels to near the normal range. The next step is taking pharmacological agents which include:-

i.Biguanides: The biguanides, of which metformin (Glucophage®) is the most commonly used, are a class of drug that functions to lower serum glucose levels by enhancing insulin-mediated suppression of hepatic glucose production, enhancing insulin-stimulated glucose uptake by skeletal muscle, increases fatty acid oxidation, and decreases the absorption of glucose from the gastrointestinal tract (Collier,Bruce, Smith, Lopaschuk, & Dyck,2006; Michael, 2016). The mode of action of metformin shows that it improves insulin sensitivity by increasing insulin

receptor tyrosine kinase activity and enhancing glycogen synthesis in hepatocytes, and by increasing recruitment and transport of GLUT4 transporters to the plasma membrane in adipose tissue. Metformin has a mild inhibitory effect on complex I of oxidative phosphorylation, has antioxidant properties, and activates both glucose-6-phosphate dehydrogenase (G6PDH) and AMP-activated protein kinase (AMPK). The importance of AMPK in the actions of metformin stems from its role in the regulation of both lipid and carbohydrate metabolism (Michael, 2016). Due to the concern of development of lactic acidosis, metformin should be used with caution in elderly diabetic individuals with renal impairment. It has a low incidence of hypoglycaemia compared to sulfonylureas (Collier, Bruce, Smith, Lopaschuk & Dyck, 2006).

ii. The sulfonylureas andmeglitinides: The sulfonylurea and meglitinide classes of oral hypoglycaemic drugs are referred to as endogenous insulin secretagogues because they induce the pancreatic release of endogenous insulin. They both function by binding to and inhibiting the pancreatic ATP-dependent potassium channel that is normally involved in glucose-mediated insulin secretion, though the binding site is different (Fuhlendorff,Rorsman, Kofod, Brand, Rolin, & MacKay 1998; Michael, 2016). The first generation sulfonylureas (tolbutamide, acetohexamide, chlorpropramide and tolazamide) are not routinely prescribed any longer in the US. The second generation sulfonylureas include glipizide (Glucotrol®), glimepiride (Amaryl®) and glyburide (DiaBeta®, Micronase®, Glynase®).These drugs have no significant effects on circulating triglycerides, lipoproteins or cholesterol (Michael, 2016).These are generally well tolerated, but because they stimulate endogenous insulin secretion, they carry a risk of hypoglycaemia (Chiniwala& Jabbour, 2011).

iii. Thiazolidinediones (TZDs): Thiazolidinediones are insulin sensitizers.They are synthetic ligands for the transcription factor peroxisomes proliferator-activated gamma (PPARγ)(Yki-Järvinen, 2004; Loghmani, 2012). TZDs function as agonists for the transcription factor, PPARγ. PPARγ is a member of the superfamily of nuclear receptor transcription factors (Michael, 2016). They are the first drugs to address the basic problem of insulin resistance in type 2 DM patients (Yki-Järvinen, 2004), whose class now includes mainly pioglitazone after the restricted use of rosiglitazone recommended by Food and Drug Administration (FDA) due to increased cardiovascular events reported with rosiglitazone (Yoon,Lee, Kim, Cho, Choi, & Ko, 2006). Pioglitazone use is not associated with hypoglycaemia and can be used in cases of renal impairment, and thus well tolerated in older adults. On the other hand, due to concerns regarding

peripheral aedema, fluid retention and fracture risk in women, its use can be limited in older adults with DM.

iv. α-Glucosidase inhibitors: α-glucosidase inhibitors such as acarbose (Precose®) and miglitol (Glyset®) function by interfering with the action of the α-glucosidase present in the small intestinal brush border. The consequence of this inhibition is a reduction in digestion and the consequent absorption of glucose into the systemic circulation. The reduction in glucose uptake allows the pancreatic β-cells to more effectively regulate insulin secretion. The advantage of the use of the α-glucosidase inhibitors is that they function locally in the intestine and have no major systemic action. Hypoglycaemia does not usually occur with the use of α-glucosidase inhibitors, but they are effective at reducing fasting plasma glucose (FPG) levels and levels of glycosylated haemoglobin (HbA_{1c}). The common adverse side effects of these inhibitors are abdominal bloating and discomfort, diarrhoea and flatulence (Loghmani, 2012; Michael, 2016).

v. Incretin-based therapies: The foundation of incretin-based therapies istargeting glucagon-like peptide-1 (GLP-1) (Stonehouse, Darsow, & Maggs, 2012).The primary metabolic responses to GLP-1 release from the enteroendocrine L-cells of the gut are inhibition of glucagon secretion and enhancement of glucose-dependent insulin release from the pancreas, but only when glucose levels are elevated (Baggio &Drucker, 2007; Michael, 2016), thus offering the potential to lower plasma glucose while reducing the likelihood of hypoglycaemia. The hormonal action of GLP-1 is rapidly terminated as a consequence of enzymatic cleavage by dipeptidylpeptidase IV (DPP IV or DPP4). Recent clinical evidence has shown that either infusion of GLP-1 or inhibition of DPP4 can result in dramatic reductions in plasma glucose concentrations, reductions in HbA_{1c} and improvement in pancreatic β-cell function (Michael, 2016). Examples are Exenatide, an incretin mimetic, and Liraglutide (Chiniwala & Jabbour, 2011).

vi. SGLT2 antagonists: A new class of orally administered compounds that targets renal glucose transport and inducers of glucosuria have been tested for efficacy in type 2 diabetes treatment (John &Wilding, 2009; Michael, 2016). Two sodium-glucose co-transporters (SGLT1 and SGLT2) have been identified as responsible for this renal glucose reabsorption. SGLT2 is expressed nearly exclusively in the cortex of the kidney and has been shown to be responsible for approximately 90% of the renal glucose reabsorption. Therefore, it is postulated that selective inhibition of the renal SGLT2 activity should result in greatly enhanced glucose release in the

urine. Several drugs (all of which carry the suffix "-gliflozin"**:** empagliflozin, canagliflozin, dapagliflozin, ipragliflozin) that inhibit SGLT2 are currently under investigation. Canagliflozin (Invokana®), dapagliflozin (Farxiga®), and empagliflozin (Jardiance®) are the only drugs in this class that have been approved by the FDA for the treatment of type 2 diabetes(John&Wilding, 2009;Michael, 2016).

vii. Insulin therapy: Insulin is used alone or in combination with oral hypoglycaemic agents. Augmentation therapy with basal insulin is useful if some beta cell function remains. Replacement of basal-bolus insulin is necessary if beta cell exhaustion occurs. Rescue therapy using replacement is necessary in cases of glucose toxicity which should mimic the normal release of insulin by the beta cells of the pancreas (Mayfield & White, 2004). Insulin comes in injectable forms - rapid acting, short acting, intermediate acting and long acting. The long acting forms are less likely to cause hypoglycaemia compared to the short acting forms.

viii. Insulin analogues: Insulin therapy was limited in its ability to mimic normal physiologic insulin secretion. Traditional intermediate- and long-acting insulins (NPH insulin, lente insulin, and ultralente insulin) are limited by inconsistent absorption and peaks of action that may result in hypoglycaemia. The pharmacokinetic profiles of the new insulin analogues are distinct from those of the regular insulins, and their onset and durations of action range from rapid to prolonged. Two rapid-acting insulin analogues, insulin lispro and insulin aspart, and one long-acting insulin analogue, insulin glargine, are available (Cameron &Bennett, 2009).

2.10 Oxidative stress in diabetes and diabetic complications

Oxidative stress results from the imbalance between oxidants production and the antioxidant capacity, a condition that favours the increase in the levels of radical species, such as superoxide anion ($O_2^{\bullet-}$), hydroxyl ($^{\bullet}OH$) and peroxyl ($ROO^{\bullet}$) radicals and non-radical species, such as hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl). These species appear as the commonest reactive oxygen species (ROS) that react with lipids, DNA and proteins in cells, leading to the loss of biological function.Chronic hyperglycaemia appears as a crucial factor in the development of oxidative stress in various tissues, mainly those in which the glucose transport is partially independent of insulin, such as pancreas, vascular endothelium, retina, kidney, and liver (Porto Dechandt, Pereira De Souza, Siqueira, Pereira, De Assis, Da Silva …& Baviera, 2014). Oxidative stress contributes to the pathogenesis of glucotoxicity during the

development of diabetes and diabetic complications (Stadler, 2012; Arora &Singh, 2014). However reductive stress due to excess reduced form of nicotinamide adenine dinucleotide (NADH) (Teodoro,Rolo & Palmeira 2013; Bassi,Trevisani, & Tezza, 2012) generated by high blood glucose followed by oxidative stress comprises the fundamental pathogenic mechanisms of chronic hyperglycaemia in the development of diabetes and diabetic complications (Liang-Jun, 2014).

The glycolytic pathway breaks down nearly 80%–90% of the body's glucose, while the pentose phosphate pathway consumes the remaining 10%–20% under physiological condition (Abdul-Ghani & DeFronzo, 2010; Wamelink,Struys, & Jakobs, 2008). Under hyperglycaemic condition, more glucose will flux through the glycolytic pathway that produces more pyruvate and acetyl-CoA, leading to more NADH production. As NADH is an electron carrier, excess amount of it will cause an electron pressure on the mitochondrial electron transport chain (Ola,Berkich, & Xu, 2006; Rosca, Vazquez, Chen, Kerner, Kern, & Hoppel, 2012). This is particularly true for hepatocytes and pancreatic β-cells in that glucokinase (hexokinase D) is a supply-driven enzyme, and this enzyme is not inhibited by glucose-6-phosphate (G6P) (Pal, 2009). Therefore, the more glucose, the more G6P produced that will be broken down through glycolysis and Krebs cycle, leading to more NADH production. The electron pressure induced by overproduced NADH will lead to a proportional increase in electron leakage that will partially reduce oxygen to yield superoxide (Hirst,King, & Pryde, 2008; Yan, 2014a). Superoxide is the precursor of all reactive oxygen species that at elevated levels can cause oxidative stress (Jackson,Papa, & Bolaños, 2002; Yan, 2014b). Superoxide can be converted to hydrogen peroxide by superoxide dismutase. Hydrogen peroxide can then be converted to form hydroxyl radical by metal ions (Jackson, Papa & Bolanus, 2002). Superoxide can also react with nitric oxide to produce peroxynitrite ($ONOO^-$) (Cai and Yan, 2013).All these reactive species can cause oxidation of proteins, lipids, and DNA. Consequently, an oxidative stress condition can fully develop due to a high level of NADH, achieving the transition from reductive stress to oxidative stress (Liang-Jun, 2014).

In addition to the elevated ROS production, reduction in the activity of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) also contributes to the development of oxidative stress in DM (Sindhu,Koo, Roberts& Vaziri, 2004).

In contrast (addition) to the above reports, a study hasdemonstrated the functional inhibition of a major cytoplasmic antioxidant system in hyperglycaemia (Schulze, Yoshioka, Takahashi, He,

King & Lee,2004). The intracellular redox balance is maintained by ROS-scavenging systems, and the two major intracellular thiol-reducing mechanisms are the interacting glutathione and thioredoxin systems (Nordberg & Arner, 2001)**.** Thioredoxin reduces ROS through reversible oxidation of thioredoxin at two cysteine residues (Cys-32 and Cys-35); thioredoxin is then reduced by thioredoxin reductase and NADPH. Thioredoxin-interacting protein (Txnip), the endogenous inhibitor of thioredoxin also known as vitamin D_3 up-regulated protein-1 (VDUP-1) (Wang,De Keulenaer & Lee, 2002) or thioredoxin-binding protein-2 (TBP-2)(Nishiyama, Matsui, Iwata, Hirota, Masutani, Nakamura . . . & Yodoi, 1999), inhibits thioredoxin antioxidative function by binding to its redox-active cysteine residues (Nishiyama *et al,* 1999; Junn,Han, Im, Yang, Cho, Um . . . & Choi, 2000). Hyperglycaemia inhibits thioredoxin ROS-scavenging function through p38 MAPK (Mitogen activated protein kinase)-mediated induction of Txnip. Overexpression of Txnip increases oxidative stress, while Txnip gene silencing restores thioredoxin activity in hyperglycaemia (Schulze*et al.*,2004).

Furthermore, increased oxidative stress appears to be a deleterious factor leading to insulin resistance, dyslipidaemia, β-cell dysfunction, impaired glucose tolerance and ultimately leading to type 2 diabetes mellitus (Tangvarasittichai, 2015). Oxidative stress has the ability to lower insulin sensitivity and damage the insulin-producing cells, the B-cells of Islets of Langerhan in the pancreas. Adipose tissues and muscle are the significant tissues participating in insulin resistance. Oxidative stress modifies the signaling pathway within a cell installing insulin resistance (Evans, Goldfine, Maddux & Grodsky, 2003).Insulin signaling is modulated by ROS/RNS (reactive nitrogen species) by two ways. On one side, in response to insulin, the ROS/RNS are produced to exert its full physiological function and on the other side, the ROS and RNS express negative regulation on insulin signaling, which results in insulin resistance, a risk factor for type 2 diabetes (Erejuwa, 2012).Thus oxidative stress can induce or result from type 2 diabetes.

Oxidative stress plays a pivotal role in the establishment of various complications observed in diabetes mellitus (Giacco & Brownlee, 2010; Madonna & De Caterina, 2011; Tiwari,Pandey, Abidi & Rizvi, 2013). Oxidative stress is higher in diabetic patients with complications than patients without complications (Mandal, Bandyopadhyay,Chakrabarti & Bandyopadhyay, 2010). As has been discussed above, overproduction of NADH can lead to overproduction of mitochondrial superoxide and other forms of ROS. These ROS can then impair the activity of

glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Giacco & Brownlee, 2010), because it is very sensitive to oxidative modifications (Zhang, Gong, Zhou, Xie, Guan & Yi, 2013; Madsen-Bouterse, Mohammad & Kowluru, 2010), due to a redox-sensitive cysteine residue at its active center (Rivera-Nieves, Thompson, Levine & Moss, 1999). Additionally, high level of NADH would also inhibit GAPDH activity (Ussher, Jaswal & Lopaschuk, 2012). Such impairments would collectively decrease the efficiency of glucose metabolism via glycolysis and Krebs cycle, inducing accumulation of glyceraldehyde 3-phosphate (G3P). Therefore, G3P and all the intermediate products above it will have to be disposed by 5 pathways that branch off the glycolytic pathways (Figure 2.2) (Rask-Madsen & King, 2013). These pathways are minor and insignificant in glucose metabolism under normoglycaemic conditions, but can become major pathways to flux high level glucose. All the five pathways have been linked to more ROS production, oxidative stress, and the pathogenesis of diabetes and diabetic complications (Kassab & Piwowar, 2012; Madonna & De Caterina, 2011).

1. The polyol pathway: The increased glucose flux through the polyol pathway decreases the intracellular levels of nicotinamide adenine dinucleotide phosphate in the reduced form (NADPH) and impairing the regeneration of the reduced glutathione (GSH), one of the most important non-enzymatic endogenous antioxidants. This is because glutathione reductase needs NADPH to regenerate GSH from GSSG (oxidized form of glutathione). As GSH level goes lower, cellular antioxidant capacity can be compromised, resulting in elevated levels of reactive oxygen species that can attack macromolecules and induce oxidative damage (Liang-Jun, 2014).

2. **Advanced glycation end products (AGEs):** High level of glucose can induce formation of methylglyoxal from glyceraldehyde 3-phosphate when GAPDH function is impaired. Methylglyoxal can modify proteins via glycation of amino groups on proteins (Queisser, Yao & Geisler, 2010). One of the major products is glycated haemoglobin (HbA1c) that has been used as a biomarker for diabetes (Koga,Murai, Morita, Saito & Kasayama, 2013; Gholap, Davies, Mostafa & Khunti,2013). Therefore, this nonenzymatic process can greatly impair protein function. Moreover, this glycation pathway is known to liberate ROS and upregulate the expression of cell surface receptor for AGEs, leading to activation of thenuclear factor-κB (*NF-κB*) signaling pathway and chronic inflammation (Vlassara & Striker, 2013).

3. The protein kinase C activation pathway: Fructose 1:6-bisphosphate can break down to dihydroxyacetone phosphate and glyceraldehyde 3-phosphate with the former being readily

isomerized to glyceraldehyde 3-phosphate under the action of triose phosphate isomerase. Accumulation of glyceraldehyde 3-phosphate can increase the synthesis of diacylglycerol that is an activator of protein kinase C (PKC). PKC activation is known to be involved in elevating the content of transforming growth factor 1(TGF-1), endothelin-1, nuclear factor B(NF-B), and vascular endothelial growth factor (Feng, Ruiz & Chakrabarti, 2013) and is also known to induce ROS production by membraneous activation of NADPH oxidase(Inoguchi, Umeda, Yu, Kakimoto, Imamura, Aoki . . . & Nawata, 2000)that catalyzes one electron reduction of molecular oxygen to form superoxide (Teshima,Takahashi & Nishio, 2014). PKC activation can also induce insulin resistance by inhibiting Akt-dependent nitric oxide synthase function (Naruse,Rask-Madsen & Takahara, 2006).

4. **The hexosamine pathway:** This pathway branches off from fructose 6-phosphate in the glycolytic pathway. Fructose 6-phosphate is the substrate of the enzyme glutamine-fructose 6-P amidotransferase (GFAT), which is the rate-limiting enzyme for this pathway. GFAT makes glucosamine 6-P from fructose 6-P and the former is further converted to UDP-N-acetylglucosamine, which is the substrate for specific O-GlcNAc transferase that catalyzes posttranslational modifications of proteins via O-GlcNAc on serine and threonine residues (Ma & Hart, 2013; Fardini, Masson & Boudah,2014). Increased glucose flux through this pathway has been shown to be involved in ROS generation and oxidative stress, and has been implicated in diabetic complications(McLarty, Marsh & Chatham, 2013; Semba,Huang, Lutty, Van Eyk & Hart, 2014). Numbers 3 and 4 processes (Figure 2.2) increase the expression and activity of many factors involved in the genesis of diabetes complications (Madonna & De Caterina, 2011).

5. **The glyceraldehyde autoxidation pathway:** This pathway also branches off from glyceraldehyde 3-phosphate in the glycolytic pathway. Glyceraldehyde 3-phosphate is formed from fructose 1:6-bisphospate by the enzyme aldose. Under certain conditions, glyceraldehyde 3-phosphate can undergo autoxidation (Mira,Martinho, Azevedo & Manso 1991), a process that can generate hydrogen peroxide and α-ketoaldehydes in diabetes mellitus (Wolff and Dean, 1987).

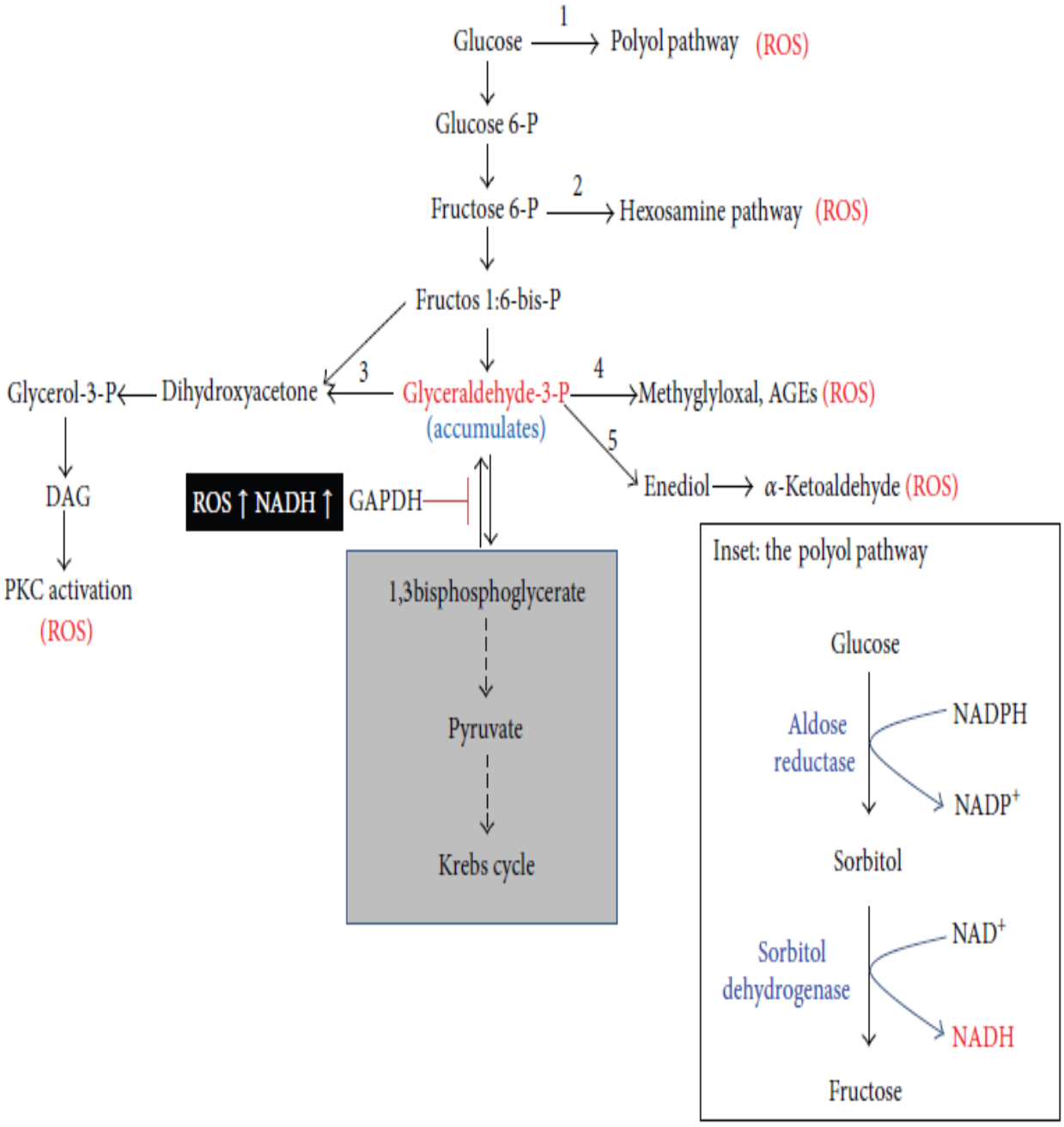

Figure 2.2: The branch-off pathways that are activated to dispose excess glucose when glyceraldehydes 3-phosphate dehydrogenase (GAPDH) is inactivated by ROS. Pathways in grey area would no longer efficiently break down glucose when GAPDH is inactivated by posttranslational modifications (Liang-Jun, 2014).

2.11 Biomarkers of oxidative stress in type 2 diabetes

The products of oxidative stress-induced alterations in major biomolecules in the cell and status of plasma antioxidants serve as biomarkers of oxidative stress during type 2 diabetes (Tiwari *et al*, 2013):-

1. Malondialdehyde (MDA): It has been reported that peroxyl radicals can remove hydrogen from lipids, producing hydroperoxides that further propagate the free-radical pathway (Lobo,Patil, Phatak & Chandra, 2010). Peroxidation of lipids produces highly reactive aldehydes, including MDA, acrolein, 4-hydroxynonenal (HNE), 4-oxononenal (ONE), and isolevuglandins (IsoLGs) (Guo, Chen, Amarnath & Davies,2012). MDA has been documented as a primary biomarker of free radical mediated lipid damage and oxidative stress (Shodehinde &Oboh, 2013). Increased MDA level in plasma, serum, and many other tissues has been reported in diabetic patients (Bandeira, Guedes, da, da Fonseca, Pires, Gelain & Moreira, 2012). Increased level of MDA in diabetics suggests that peroxidative injury may be involved in the development of diabetic complications. The increase in lipid peroxidation is also an indication of decline in defence mechanisms of enzymatic and nonenzymatic antioxidants (Saddala, Thopireddy, Ganapathi & Kesireddy, 2013). Increased lipid peroxidation presents a close relationship with the high glycaemic levels and oxidative stress in diabetes mellitus (Bandeira, *et al.*, 2012).

2. Glutathione: Glutathione (GSH), a tripeptide, γ-L-glutamyl-L-cysteinylglycine, is present in all mammalian tissues at 1-10mM concentrations (highest concentration in liver) as the most abundant non-protein thiol that defends against oxidative stress (Lu, 2013). Plasma GSH/GSSG showed a significant decrease in type 2 diabetes as compared to normal (Calabrese, Cornelius & Leso, 2012). Decreased GSH level may be one of the factors in the oxidative DNA damage in type 2 diabetics (Dinçer, Akçay, Alademir & Ilkova, 2002). Abnormal GSH status is involved in β-cell dysfunction and in the pathogenesis of long-term complications of diabetes. Uncontrolled type 2 diabetes has severely deficient synthesis of GSH attributed to limited precursors (amino acids) availability (Sekhar, Mckay, Patel, Guthikonda, Reddy, Balasubramanyam& Jahoor, 2011).

3. Catalase(CAT): Catalase is an antioxidative enzyme present nearly in all living organisms. Catalase acts as main regulator of hydrogen peroxide metabolism (Takemoto, Tanaka & Iwata, 2009). Catalase enzymatically processes hydrogen peroxide into oxygen and water, and thus

neutralizes it. Increased risk of diabetes has been documented in patients with catalase deficiency. The deficiency of this enzyme increases oxidative stress and ultimately causes failure of the β-cell. β-cells are rich in mitochondria, and thus this organelle might be a source of ROS (Góth and Eaton, 2000). Catalase protects pancreatic β-cells from damage by hydrogen peroxide. Deficiency of catalase increases mitochondrial ROS and fibronectin expression in response to free fatty acids, which were effectively restored by catalase overexpression or N-acetyl cysteine (Hwang, Lee, Huh, Park, Lee, Ho & Ha,2012). Low catalase activities together with redox active metal ions, produce highly toxic hydroxyl radicals (Góth &Bigler, 2007). Patel and coworkers reported that hyperglycaemia increases hydrogen peroxide production, hyperpolarizes mitochondrial membrane, and downregulates CAT gene expression (Patel, Chen, Das & Kavdia, 2013).

4. Superoxide dismutase: Superoxide dismutase (SOD) is the antioxidant enzyme that catalyses the conversion of superoxide anion (O_2^-) into hydrogen peroxide and molecular oxygen (Wang, Tao & Hai, 2012), and in the presence of other enzymes (e.g. Catalase) into oxygen and water (Davari,Talaei, Alaei & Salami, 2013). SOD plays important protective roles against cellular and histological damages that are produced by ROS (Davari *et al.*, 2013). Decline in the level of SOD in diabetic tissue and blood has been reported in many studies (Shukla, Dikshit, Tyagi, Shukla & Gambhir,2012; Kim, 2013; Manjulata *et al.*, 2013). Kim (2013) reported that diabetic skin tissues express a relatively small amount of extracellular protein and concluded that extracellular SOD is related to the altered metabolic state in diabetic skin, which elevates ROS production (Kim, 2013). All mammalian tissues contain three forms of SOD: Cu-Zn-SOD, Mn-SOD, and extracellular EC-SOD, and each of them is a product of a distinct gene (Li, Xia & Förstermann, 2012). Cu-Zn-SOD or SOD 1 (EC 1.15.1.1) is localized in cytosol, Mn-SOD or SOD 2 (EC 1.15.1.1) in mitochondria, and EC-SOD or SOD 3 (EC 1.15.1.1) in extracellular space (Zelko, Mariani & Folz,2002).Down-regulation of renal SOD1 and SOD3 may play a key role in the pathogenesis of diabetic nephropathy (Fujita, Fujishima, Chida, Takahashi,Qi, Kanetsuna . . . &Takahashi, 2009).

5. Glutathione peroxidase(GPx): GPx is also a biomarker of oxidative stress in type 2 diabetes. There is a significant decrease in its activity in patients with diabetes. The low activity of GPx could be directly explained by the low content of GSH found in patients with type 2 diabetes,

since GSH is a substrate and cofactor of GPx. Enzyme inactivation could also contribute to low GPx activity. GPx is a relatively stable enzyme, but it may be inactivated under conditions of severe oxidative stress. Inactivation of this enzyme may occur through glycation governed by prevailing glucose concentration (Kewal, 2010; Gawlik, Naskalski, Fedak, Pawlica-Gosiewska, Grudzień, Dumnicka . . . & Solnica, 2016).

6. Other biomarkers of oxidative stress in type 2 diabetes mellitus: Other biomarkers in type 2 diabetes mellitus oxidative stress include:

- Oxidative and glycoxidative protein damage products which are advanced oxidation protein products (AOPPs) and advanced glycation end products (AGEs) such as glycated haemoglobin and fructosamine levels(Tiwari*et al.*, 2013).
- Other antioxidants markers may include total antioxidant capacity (TAC), ferric reducing ability of plasma (FRAP)), glutathione reductase (GR), *γ*-glutamyltransferase (GGT) and uric acid in serum, and plasma and/or haemolysate levels (Gawlik *et al.*, 2016).
- Antioxidant vitamins; vitamins A, C and E which act by detoxifying the free radicals (Asmat, Abad & Ismail2015).

2.12 Dyslipidaemia in type two diabetes mellitus

Dyslipidaemia (lipid abnormalities or abnormal changes in lipid profile) is an important feature of type 2 diabetes. These abnormalities are known to be risk factors for the development of atherosclerosis and have been described as predictors of CHD (coronary heart diseases) in type 2 diabetes. Reduced action of insulin at the tissue level explains many of the lipid abnormalities in type 2 diabetes, although relative insulin deficiency associated with pancreatic beta-cell dysfunction and other factors (e.g. increased dietary fat consumption) may also contribute (Vergès, 2015; Kumar,Dey, Suresh, Chaudhuri, Panda, Mitra & Hazra, 2014).Common lipid abnormalities during diabetes induced dyslipidaemia include: - decreased HDL-c and apoproteinA-1(apoA-1), increased VLDL, elevated apoB and small dense LDL-c, hypercholesterolaemia, hypertriglyceridaemia, and slightly elevated LDL-c. (Philip, 2008; Kumar *et al.*, 2014).

-**Hypertriglyceridaemia:** Reduced action of insulin on adipocytes (due to insulin resistance) results in reduced suppression of lipolysis (hydrolysis of stored triglyceride) and so greater release of non-esterified fatty acids (NEFA) which is transported to the liver. Type 2 diabetes is

associated with visceral adiposity, so increasing delivery of NEFA into the portal circulation and so pass directly to the liver, stimulating synthesis of triacylglycerols in hepatocytes.The increased hepatic triglycerides are packaged into lipoprotein particles containing apolipoprotein B-48 (apoB-48; chylomicrons) and apolipoprotein B-100 (apoB-100; very-low density lipoprotein, VLDL). Thus,hypertriglyceridaemia potentially results from increased production of VLDL, reduced clearance of triglyceride-rich lipoproteins (TRL) (VLDL and chylomicrons) or a combination of both mechanisms. The reduced clearance of TRL isas a result of reduced lipoprotein lipase (LpL) activity(water-soluble enzyme, that hydrolyzes triglyceride rich lipoproteins-) which is regulated by insulin, and its action is decreased in type 2 diabetes(Alan, 2012;Pang, Chan& Watts,2014).

-Low HDL-c concentrations: The reductions in HDL associated with type 2 diabetes and insulin resistance are multifactorial, with each factor resulting in increased catabolism of HDLs. Hypertriglyceridaemia is a major contributing factor to the accelerated HDL catabolism observed in type 2 diabetes. In the presence of hypertriglyceridemia, increased concentrations of plasma VLDL promote the cholesterol ester transfer protein (CETP)-mediated exchange of VLDL triglyceride for HDL cholesteryl esters(Goldberg, 2001). Clinical measurements of HDL are of HDL cholesterol; therefore, substitution of triglyceride for cholesteryl ester in the core of the particle leads to a decrease in this measurement. Moreover, the triglyceride-rich HDL particles are very good substrates for plasma lipase, especially hepatic lipase that converts HDL to smaller particles that are more rapidly cleared from the plasma .The surface lipid from triglyceride-rich particles that are transferred to HDL during VLDL and chylomicron lipolysis increases HDL lipid content. This pathway of HDL production is therefore decreased in the insulin resistant state due to decreased lipoprotein lipase activity (Goldberg, 2001;Vergès, 2015).

-Increased VLDL-c: Insulin resistance is associated with reduced inhibition of hormone-sensitive lipase in adipose tissue by insulin, leading to increased lipolysis and, thereby, augmented NEFA portal flux to the liver. In diabetes, greater amounts of fatty acids returning to the liver increases hepatictriglyceride production which in turn serves to drive hepatic VLDL production. Furthermore, the normal suppressant effect of insulin on postprandial VLDL (more specifically, $VLDL_1$) production is blunted by hepatic insulin resistance (Sondergaard, Sorensen, Rahbek, Gormsen, Christiansen & Nielsen, 2012). Both increased production and delayed catabolism of VLDL are responsible for the increased VLDL pool. Moreover not all VLDL are

equally likely to be converted to LDL. A greater proportion of large (due to greater triglyceride content) lighter VLDL return to the liver without complete conversion to LDL (Goldberg, 2001;Vergès, 2015).

-Increased small dense LDL: In patients with type 2 diabetes, the mean LDL-cholesterol level is comparable or slightly elevated relative to that in individuals without diabetes. In part, this may represent a balance of factors that affect LDL production and catabolism. A necessary step in LDL production is hydrolysis of its precursor VLDL by LpL. A reduction in this step due to LpL deficiency or excess surface apoproteins (C1, C3, or possibly E) decreases LDL synthesis. Conversely, increases in this lipolytic step that accompany weight loss, fibric acid drug therapy, and treatment of diabetes may increase LDL levels. LDL particles that are triglyceride enriched due to the hypertriglyceridaemia and increased CETP activity, are also converted by the triglyceride lipase activity of hepatic lipase into preferential smaller and denser particles over larger ones(Goldberg, 2001;Vergès, 2015).Small dense LDL particles are more inclined to oxidation, and, particularly in type 2 diabetes, by glycation, and are more atherogenic (Valabhji & Elkeles, 2003;Roever,Casella-Filho, Dourado, Resende & Chagas, 2014).

2.13 Atherogenic indices:

In type 2 diabetic patients, cardiovascular disease (CVD) is the cause of morbidity and mortality. Though the pathogenesis of CVD in diabetes is multifactorial, atherogenic dyslipidaemia is found to be the most powerful risk factor. The use of LDL-c alone for assessment of cardiovascular risk would ignore the TG-rich lipoproteins. Non-HDL cholesterol (TC- HDL-c), lipid ratios (TC/HDL-c, and LDL-c/HDL-c, atherogenic coefficient and atherogenic index of plasma) have been found to indicate an atherogenic risk and are better predictors of CVD, hence their use in assessing the CVD risk in type 2 diabetes mellitus. The ratios contribute significantly to the estimation of CVD risk in type 2 diabetes mellitus especially, when the absolute values of lipid profile seem normal or not markedly deranged (Adu, Ukwamedu & Oghagbon, 2015;Nimmanapalli, Kasi, Devapatla & Nuttakki, 2016).

Non-HDL cholesterol (TC- HDL-c): The amount of LDL-c inside the lipoprotein particle varies in individuals, hence its measurement does not reflect the number of particles and therefore the true level of cardiovascular risk. A more precise way to determine risk would be to measure the number of atherogenic lipoprotein particles in the serum that is apolipoprotein-B. Non-HDL

cholesterol (TC- HDL-c) is indicator of atherogenic apolipoprotein B containing lipoproteins such as LDL, VLDL (very low density lipoproteins) and intermediate density lipoproteins (IDL). A report confirmed that non-HDL cholesterol (TC-HDL-c) is a better predictor of CVD risk than a simple measure of LDL cholesterol (Peters, 2008).

Castelli's risk index1 (CRI-I) and 2 (CRI-II): Studies revealed that rather than the cholesterol concentration in different lipoproteins, the size and composition are found to be important in atherogenesis. Lipid ratios have also been found to indicate an atherogenic risk and are said to be better predictors of coronary artery diseases than lipids alone. Hence lipid parameters can be combined into ratios that reflect the proportion of atherogenic to anti atherogenic lipids and lipoproteins. Proposed lipid ratios for CV risk assessment include TC/HDL-c, TG/HDL-c, and LDL-c/HDL-c (Nimmanapalli *et al.,* 2016). The use of ratios such as TC/HDL-c (Castelli's risk index (CRI-I) or LDL-c/HDL-c (Castelli's risk index (CRI-II) may provide refined risk assessment for cardiovascular outcomes by simultaneously taking into account both atherogenic and cardioprotective lipid fractions(Mohieldein,Abdalla & Hasan, 2014).The ratio of TC/HDL-c is regarded as a predictor of CHD risk, especially with values >6.0 (Gordon,Ragoobirsingh, Morrison, Choo-Kang, McGrowder & Martorell, 2010), while a cut-off value for a high LDL-c/HDL-c ratio was defined as 3.5(Shimomura & Wakabayashi, 2013).

Atherogenic coefficient: Atherogenic coefficient (AC) calculated as {(TC- HDLc)/HDLc} or (non-HDL-c)/HDL-c) is yet another ratio relying on the significance of HDLc in predicting the risk of cardiovascular diseases. AC is a measure of cholesterol in LDL, VLDL, and IDL (intermediate density lipoproteins) fractions with respect to HDLc (good cholesterol) (Bhardwaj, Bhattacharjee, Bhatnagar &Tyagi,2013; Nimmanapalli *et al.,* 2016).

Atherogenic index of plasma: Among the lipoprotein subclasses, disproportionate amounts of small, dense LDL particles and small HDL particles constitute atherogenic profile due to a high susceptibility to oxidation. However, as the sub fractionation of lipoproteins cannot be undertaken in all the clinical laboratories, the atherogenic index of plasma (AIP) calculated according to the formula, log (TG/HDL-c), has been shown to correlate with the size and composition of lipoproteins. Also, the ratio log (TG/HDL), correlates well with the size of HDL and LDL particles and with the fractional esterification rate of cholesterol by lecithin: cholesterol acyl transferase in plasma (Nimmanapalli *et al.,* 2016). It has been shown that atherogenic index of plasma (AIP) is a strong marker to predict the risk of atherosclerosis and coronary heart

disease (Nwagha,Ikekpeazu, Ejezie, Neboh & Maduka, 2010; Tariq & Ali, 2012). AIP reflects the true relationship between protective and atherogenic lipoprotein and is associated with the size of pre- and anti- atherogenic lipoprotein particles (Dobiášová,Frohlich, JŠedová, Cheung & Brown,2011). It has been suggested that an AIP value of under 0.11 is associated with low risk of CVD; the values between 0.11 to 0.21 and above 0.21 are associated with intermediate and increased risks respectively (Dobiasova, 2006; Dobiášová *et al,* 2011).

2.14 Anthropometric indices

Anthropometry is the measurement of size and proportions of the human body. Anthropometric parameters are commonly used as research tools to assess the non-communicable disease risk factors in populations as they are easy to implement, inexpensive, and valid. However, the threshold cut off values varies from population to population (Himabindu, Sriharibabu, Alekhya, Saisumanth, Lakshmanrao&Komali,2013). The body mass index (BMI), waist circumference (WC), waist to hip ratio (WHR) and waist to height ratio (WHtR) are the main anthropometric parameters for predicting incidence of type 2 diabetes in populations (Lotfi, Saadati & Afzali, 2014; Nahar,Dubey, Joshi, Phadnis & Sharma, 2012).Also mid arm circumference (MAC) is an alternate anthropometric index of obesity in type 2 diabetes (T2D) and therefore can predict the onset of T2D (Devang, Nandini, Rao & Adhikari,2016).

The body mass index (BMI):BMI has traditionally been the chosen indicator to measure body size and composition (muscle, fat, and bone), and to diagnose underweight (below 18.5 kg/m^2), normal weight (18.5 to 25 kg/m^2), overweight (25 to 30 kg/m^2), or obese (over 30 kg/m^2) (WHO**,** 2012).Overweight and obese individuals are at an increased risk of type 2 diabetes and its complications, including CVD (Huxley, Mendis, Zheleznyakov, Reddy & Chan,2010).

Waist circumference (WC), waist–hip ratio (WHR) and waist–height ratio (WHtR): These are alternative measures that reflect abdominal adiposity (rather than overall adiposity as in the case of BMI) and have been suggested as being superior to BMI in predicting CVD risk. This is based largely on the rationale that increased visceral adipose tissue is associated with a range of metabolic abnormalities, including decreased glucose tolerance, reduced insulin sensitivity and adverse lipid profiles, which are risk factors for type 2 diabetes and CVD (WHO, 2011). According to the US National Institutes of Health (NIH) waist circumference in excess of 102 centimetres for men and 88 centimetres for non-pregnant women is considered to be high risk for

type 2 diabetes, dyslipidaemia, hypertension, and CVD (NIH, 2016). WHtR may be a better predictor of CVD risk factors, because it takes into account differences in height, unlike WC which does not account for differences in height, therefore may potentially over- and under-evaluate risk for tall and short individuals respectively (Browning, Hsieh & Ashwell, 2010).

Mid arm circumference (MAC): MAC is an alternative index to measure obesity instead of waist circumference and BMI having shown a significant correlation with BMI and WC. MAC is clinically advantageous because it is quick, portable and inexpensive, uncomplicated and noninvasive and can be measured without difficulty. Also it can be performed on most weakened patients whose weight or height cannot be easily taken (Cicek, Ozturk, Mazicioglu, Inanc & Kurtoglu,2010; Devang *et al.*, 2016).

2.15Blood pressure(BP) and type 2 diabetes

Diabetes adversely affects the arteries, predisposing them to atherosclerosis (narrowing of the arteries), which consequently cause high blood pressure, and if not treated, can lead to further blood vessel damage, and stroke, heart failure, heart attack or kidney failure. Therefore uncontrolled diabetes increases the risk of developing high blood pressure and other cardiovascular problems (Bernard & Chao,2012; WebMD, 2016). About 80% of people with type 2 diabetes have high blood pressure (Blood pressure UK, 2008). BP of above 140/90 mmHg is considered hypertensive (AHA, 2014). The BP should not be above 130/90 mmHg for diabetic patients, although >80mmHg (diastolic) may still be risky for certain diabetic patients (ADA, 2016).

Research has shown that diabetes and hypertension share common pathways such as obesity, inflammation, oxidative stress, and insulin resistance. Hypertension and diabetes are both end results of metabolic syndrome. They may, therefore, develop one after the other in the same individual (Bernard& Chao,2012).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1. Chemicals

- HDL-c precipitant (phosphotungstic acid and Magnesium chloride), RANDOX Laboratories Ltd., Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom, BT29 4QY.
- Triglycerides Reagent(Glycerol phosphate oxidase/peroxidase), BioSystems, C/Costa Brava 30 08030 Barcelona, Spain)
- Cholesterol Reagent (Cholesterol oxidase/peroxidase), BioSystems.
- Glucose oxidase Reagent, RANDOXLaboratories Ltd., United Kingdom
- Reduced glutathione, epinephrine, DTNB (5-5'-dithiobis-2-nitrobenzoic acid), TBA (thiobarbituric acid), and hydrogen peroxides, Sigma Aldrich, Germany.
- TCA (Trichloroacetic acid), sodium dihydrogen phosphate, disodium hydrogen phosphate, SSA (Sulfosalicyclic Acid), Sodium hydrogen bicarbonate, sodium carbonate, sodium hydroxide, and ammonium molybdate, BDH, England.

3.1.2 Equipment

- Table Top Medical Lab Centrifuge 80-3, Wincom Company Ltd., China.
- Biochemistry auto-analyzer, Prietest Easylab, ACUREX, US
- Spectrophotometer APAL PD303S, Japan.
- Incubator MEMMERT, Germany.
- Waterbath with shaker ThermoScientific 2871, USA.

3.1.3 Subjects

Human subjects clinically diagnosed with Type 2 diabetes mellitus at Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Awka; andapparently non-diabetic individuals were used in this study

3.2 Methods

3.2.1 Study population: The study was carried out at Chukwuemeka Odumegwu Ojukwu University Teaching Hospital (COOTH), Awka**(**Figure 3.1). Awka isthe capital city of Anambra State in the tropical rain forest zone of Nigeria. It is located between two major cities in Northern Igboland namely Onitsha and Enugu. The longitudinal and latitudinal location of Awka is 6 12N, 7 5E in the Nigerian Cities place category (Blexxonmak (m), 2010).

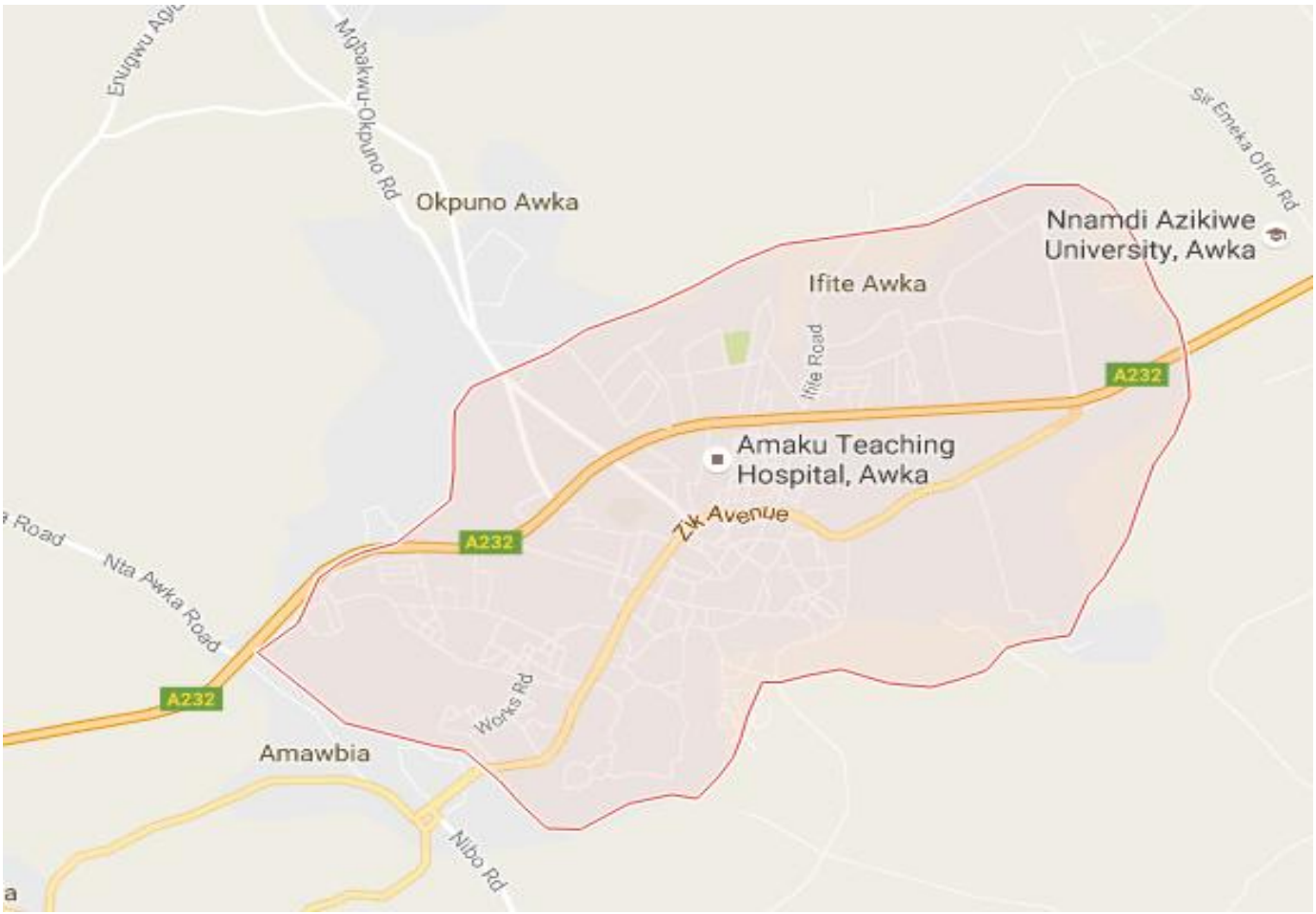

Figure 3.1: Map of Awka showing COOUTH Source: Google, 2016

3.2.2 Selection of human subjects

A total number of 100 individuals were enrolled into the study, out of which 60 (30males and 30females) were clinically diagnosed type 2 DM patients and 40 (20 males and 20 females) were

apparently healthy non-diabetic control individuals. All subjects were of age bracket 40-70 years. This study was approved by the hospital ethical committee(COOUTH/AA/VOI.I.012).All the subjects were fully informed about the nature and the purpose of the study. Written informed consent was also obtained from them.

Inclusion criteria

Subjects were included into the study if they were:

- Aged 40 to 70 years
- Willing to give their informed consent.
- Diabetic patients with fasting blood sugar ≥ 126mg/dl.

Exclusion criteria:

Subjects were excluded from the study if they:

- Have chronic infections like HIV and TB.
- Havegastrointestinal tract infection
- Were smokers or alcoholics
- Were pregnant women
- Unwilling to give written consent
- Subjects on vitamin drugs

3.2.3 Experimental design

The human subjects were divided into 2 groups (diabetic and non-diabetic).The male and female experimental units were considered homogenous and later heterogeneous to study variations in their biochemical parameters according to sex.

3.2.4 Sample collection and preparation

Venous blood samples (5 ml) were collected by venipuncture from diabetic and normal human subjects in the morning after an overnight (i.e. after 12 hour) fast, into covered sterile gel activator vacuum tubes, and allowed to clot by leaving it undisturbed for 15-30 minutes at room temperature. Then the serum was separated from the clot by centrifuging at1795xgfor 10 minutes in a centrifuge. The supernatant (serum) was immediately transferred into a clean plain tube. The samples were stored at -20°C until used for the analysis of the lipid profile and oxidative stress parameters. Note: The blood sample for serum glucose determination was collected into sodium

fluoride (NaF) tubes and the serum separated immediately was used for fasting blood glucose analysis.

3.2.5Baseline information of the Diabetic Subjects:

Information about the subjects on demographic data, nutrition/life style and medical history were obtained through interview questionnaire (Appendix I).

3.2.6Determination of fasting blood sugar (FBS)

The glucose concentration was measured by the glucose oxidase methodaccording to the method of Barham & Trinder (1972).

Principle: Glucose oxidase catalyses the oxidative transformation of β D- glucose to D-glucono1,5 lactone with the formation of hydrogen peroxide. The lactone is slowly hydrolysed to D-gluconic acid. The hydrogen peroxide formed reacts, under catalysis of peroxidase, with phenol and 4-aminophenazone to form a red - violet quinoneimine dye as indicator.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{Glucose oxidase}} \text{Gluconic acid} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{phenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4H_2O$$

Procedure: Exactly 1000µl each of Randox glucose reagent was added to three test tubes labelled -blank, standard (containing 10µl glucose standard) and sample (containing 10µl serum).The contents of the tubes were mixed, incubated for 25 minutes at25^{o}C. The absorbance of the standard (A standard) and the sample (A sample) were measured against the reagent blank within 60 minutes, at 500nm.

Calculation:

Glucose Concentration =Asample/Astandard × 100 (mg/dl), where A = absorbance

3.2.7 Lipid profile

3.2.7.1 Determination of total cholesterol

Serum total cholesterol was determined according to the method of Allian, Poon, Chan, Richmond & Fu(1974).

Principle: Cholesterol esters are hydrolyzed to free cholesterol by cholesterol ester hydrolase(cholesterol esterase) (EC 3.1.1.13). The free cholesterol produced is oxidized by cholesterol oxidase to the ketone cholest-4-en-3-one with simultaneous production of hydrogen

peroxide, which oxidatively couples with 4-aminoantipyrine and phenol in the presence of peroxidase to yield a chromogen with maximum absorption at 500nm.

Cholesterol ester+ $H_2O \xrightarrow{\text{Chol.esterase}}$ Cholesterol + Fatty acid

Cholesterol + ½0_2 + $H_2O \xrightarrow{\text{Chol.oxidase}}$ Cholestenone + H_2O_2

$2H_2O_2$ + 4-aminoantipyrine + phenol $\xrightarrow{\text{Peroxidase}}$ Quinoneimine + $4H_2O$

Procedure: Exactly 1.0mL of a single reagent (containing cholesterol esterase 0.2U/mL, cholesteroloxidase 0.1U/mL, peroxidase 0.8U/mL, phenol 28mmol/L, 4-aminoantipyrine 0.5mmol/L, Pipes 35mmol/L and sodium cholate 0.5mmol/L) was pipetted into three labeled test tubes -blank, standard (containing 10µL cholesterol standard), and sample(containing 10µL of serum).The contents were thoroughly mixed and incubated at room temperature for 10 minutes.The absorbance of the standard and the samples were measured against the blank using a Biochemistry Auto-analyzer which directly displayed the total cholesterol concentration in mmol/L.

3.2.7.2 Estimation of HDL-cholesterol

Principle: Very low density lipoprotein (VLDL), low density lipoprotein (LDL) and chylomicron fractions in serum or plasma are separated from HDL by quantitative precipitation on addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol in the HDL fraction which remains in the supernatant is analysed with enzymatic cholesterol method described in section 3.3.2.1 above.This is based on the method of Lopes-Virella, Stone, Ellis, & Coiweil (1977).

Procedure: Exactly 200µLeach of serum sample and standard were added into two different centrifuge tubes labeled accordingly. To these 500µLeach of diluted HDL-c precipitant were added, well mixed and allowed to stand for 10 minutes at room temperature. Then they were centrifuged for10 minutesat 4,000rpm.The clear supernatant was immediately separated and the cholesterol content determined.

3.2.7.3 Determination of Triacylglycerol:

Kinetic enzymatic method was used to determine triacylglycerolbased on the method by Fossati & Prencipe (1982).

Principle: Serum triacylglycerol is hydrolyzed by lipase, and the released glycerol is estimated in a reaction catalyzed by glycerol kinase and L-alpha -glycerol-phosphate oxidase in a system that generates hydrogen peroxide. The hydrogen peroxide produced oxidatively couples with 4-

aminoantipyrine and phenol in the presence of peroxidase to yield a chromogen with maximum absorption at 500nm.

Triglycerides + H_2O $\xrightarrow{\text{Lipase}}$ Glycerol + Fatty acids

Glycerol + ATP $\xrightarrow{\text{glycerol kinase}}$ Glycerol – 3– p + ADP

Glycerol – 3– p + 0_2 $\xrightarrow{\text{G-3-P- oxidase}}$ Dihydroxyacetone – P + H_2O_2

$2H_2O_2$ + 4-aminoantipyrine + phenol $\xrightarrow{\text{Peroxidase}}$ Quinoneimine + $4H_2O$

Procedure: Exactly 1.0 mL each of triacylglycerolreagent was pipetted into three labeled test tubes -blank, standard (containing 10µLtriacylglycerolstandard), and sample (containing 10µL serum).The contents were thoroughly mixed and incubated at room temperature for 15 minutes. The absorbance of the standard and the samples were measured against the blank using a Biochemistry Autoanalyzer at 500nm which displayed the triacylglycerolconcentration directly in mmol/L.

3.2.7.4 Estimation of VLDL-cholesterol and LDL-cholesterol:

The formula published by Friedewald, Levy & Fredricksonin 1972 and validated by Warnick, Knopp, Fitzpatrick & Bransonin 1990 was used to calculate VLDL-c and LDL-c concentrations using the total cholesterol, triacylglyceroland HDL-c concentrations directly estimated using reagents.

VLDL-c (mmol/L) = triacylglycerol/2.2

LDL-c (mmol/L) = Total cholesterol – (HDL-c + VLDL-c)

3.2.8 Atherogenic indices

The atherogenic indices were calculated as described below.

1. Non-HDL cholesterol = TC- HDL-c (Ram, Ahmed, Hashmi & Jabbar, 2014).
2. Castelli's Risk Index1 (CRI-I) = TC/HDL-c (Mohieldein *et al.,* 2014).
3. Castelli's Risk Index 2 (CRI-II) = LDL-c /HDL-c (Mohieldein *et al.,* 2014).
4. Atherogenic Coefficient (AC) = (TC- HDL-c)/HDL-c or (non-HDL-c)/HDL-c) (Bhardwaj*et al,* 2013; Nimmanapalli *et al.,* 2016).
5. Atherogenic Index of Plasma = Log (TG/HDL-c) (Nimmanapalli *et al.,* 2016).

3.2.9 Oxidative stress markers

3.2.9.1 Determination of reduced glutathione (GSH)

Reduced glutathione was determined by the method of Jollow, Mitchell, Zampaglione & Gillette (1974).

Principle: Yellow colour is developed when a thiol reagent, 5-5'-dithiobis-2-nitrobenzoic acid (DTNB), is added to a compound containing sulphydryl groups.The colour developed is read at 412nm in spectrophotometer.

Procedure: The sample (0.3 ml) was precipitated with 0.3 ml of sulfosalicylic acid (4%), and kept at 4°C for 1 hour and then centrifuged at 1200 g for 20 min at 4°C. The assay mixture contained 0.1 ml filtered aliquot, 2.7 ml phosphate buffer (0.1 M, pH 7.4) and 0.2 ml DTNB in a total volume of 3.0 ml. The yellow colour developed was read immediately at 412 nm agaist the reagent blank containing sulfosalicylic acid (SSA) in place of serum, and 40 umol/l of reduced glutathione was used as standard.

Calculation: Conc. of GSH (umol/l) = OD_{test}/OD_{std} X standard concentration.

3.2.9.2 Assay of superoxide dismutase (SOD) Activity

The SOD activity was assayed by the method of Misra & Fredovich(1972).

Principle: The ability of superoxide dismutase to inhibit the auto oxidation of adrenaline at pH 10.2 makes this reaction a basis for the SOD assay. Superoxide anion (O_2^-) generated by the xanthine oxidase reaction is known to cause the oxidation of adrenaline to adrenochrome. The yield of adrenochrome produced per superoxide anion introduced increases with increasing pH and also with increasing concentration of adrenaline. These lead to the proposal that auto oxidation of adrenaline proceeds by at least two distinct pathways,one of which is a free radical chain reaction involving superoxide radical and hence could be inhibited by SOD.

Procedure:

Exactly 80µl of sample was added to 1000µLof carbonate buffer (pH10.2), mixed thoroughly, and allowed to equilibrate by incubating at 37 °C for 5 minutes. Then 600µL of freshly prepared epinephrine was added and the reaction mixture was read at 30 seconds interval for 150 seconds at 480 nm. The blank was treated the same way except that 80µl of distilled water was used instead of serum. The changes in absorbance of both test and blank were determined. The %

inhibition of auto oxidation of epinephrine by SOD was calculated and the serum SOD activity was expressed as U/ml. One unit of SOD activity was equivalent to the amount of SOD that can cause 50% inhibition of epinephrine.

Calculation:

% inhibition = $(\Delta OD_{blank} - \Delta OD_{test} / \Delta OD_{blank})$ X 100

Enzyme Unit (U/ml) = (% inhibition/50) X dilution factor

3.2.9.3 Determination of malondialdehyde (MDA)

MDA was determined using the method of Gutteridge &Wilkins(1982).

Principle: MDA is a product of lipid peroxidation. When heated with thiobarbituric acid(TBA) under alkaline condition, it forms a pink coloured product, which has absorption maximum at 540nm.

Procedure: To 0.1 ml of sample in test tube was added 1 ml of 1% Thiobarbituric acid dissolved in alkaline medium (sodium hydroxide). The mixture was mixed thoroughly, and 1 ml of glacial acetic acid was added to the mixture. The reaction mixture was also shaken thoroughly and incubated in boiling water (100 °C) for 15 minutes. It was allowed to cool and the turbidity removed by centrifugation at 3000 rpm for 10 minutes. Thereafter, the supernatant was read at 532 nm. The same volume of TBA and glacial acetic acid was added to the blank, but 0.1 ml of distilled water was added to the blank instead of serum. The level of MDA in the serum was expressed as nmol/ml using the molar extinction coefficient for MDA (1.56x105 M-1cm-1).

Calculation:MDA (nmol/ml) = (OD X 1000000)/ E_{532}

Where E_{532} = Molar extinction coefficient for MDA ($1.56x10^5$ $M^{-1}cm^{-1}$)

3.2.9.4 Assay of glutathione peroxidase (GPx)

The activity of GPx was determined with the method of Rotruck, Pope, Ganther, Swanson,Hafeman & Hoekstra (1973).

Principle:GPx in the presence of H_2O_2 oxidizes reduced glutathione (GSH) to form H_2O. The amount of GSH consumed is directly proportional to the activity of GPx and it is expressed as U/ml (μmol of GSH consumed/minute). The GSH remaining after the reaction is allowed to react with 5-5'-dithiobis-2-nitrobenzoic acid (DTNB) to form a yellow complex that absorbs maximally at 412nm.

$2GSH + H_2O_2 \xrightarrow{GPx} GSSG + 2H_2O$

Procedure: The reaction mixture contained 0.4ml of phosphate buffer (pH 7.0), 0.1ml sodium azide and 0.2ml of the enzyme preparation (serum), 0.2ml of glutathione and 0.1ml of H_2O_2. The mixture was incubated at 37^0C for 10 minutes and the reaction was arrested by the addition of 0.4ml of 10% TCA.The tubes were centrifuged at 4000 rpm for 5 minutes. Then 0.5ml of the supernatant, 2ml of phosphate buffer(pH7.0) and 0.5ml of 40mM DTNB was mixed and the resulting yellow colour was read at 412nm wavelength. A blank was treated the same way except that it contained 0.2ml of distilled water instead of the sample. Also 20mg/100ml of GSH standard (0.651µmol/ml) was used. The activity of GPx was expressed as U/mL of serum (µmoles of GSH utilized/minute).

Calculation:

Actual Test OD = OD Blank – OD Test

Actual Std. OD = OD Std – OD Blank.

GPx activity = Actual OD Test/Actual OD STD X STD Concentration (U/mL).

3.2.9.5 Catalase activity assay

The activity of catalase was determined by the method of Hadwan & Abed (2016).

Principle: Catalase catalyzes the following reaction:

$2H_2O_2 \xrightarrow{Catalase} 2H_2O + O_2$

Thus the method is based on the reaction of undecomposed hydrogen peroxide with ammonium molybdate to produce a yellowish colour, which has a maximum absorbance at 374 nm. Catalase activity is assessed by incubating the enzyme sample in 1.0 ml substrate (65 mmol/ml hydrogen peroxide in 60 mmol/l sodium–potassium phosphate buffer, pH 7.4) at 37 °C for three minutes. The reaction was stopped with ammonium molybdate. Absorbance of the yellow complex of molybdate and hydrogen peroxide is measured at 374 nm against the blank.

Procedure: Four clean test tubes were arranged as follows: Test, control-test, standard and blank respectively. Then 0.1 ml of serum was added to test and control-test tubes, while 0.1 ml of distilled water was added to standard and blank tubes. Thereafter, 1 ml of distilled water was added to control-test and blank tubes, while 1 ml of hydrogen peroxide was added into test and standard tubes. The tubes were incubated at 37 °C for 3 minutes. Finally, 4 ml of ammonium

molybdate was added to all the tubes and the changes in absorbance were recorded at 374 nm against the reagent blank.

Calculation:

The rate constant of a first-order reaction (k) equation was used to determine catalase activity:

Catalase activity of test in kU/L = 2.303/t x (log A°/A-A^c) x Vt/Vs

Where: t = time, A° = absorbance of standard tube, A = absorbance of test tube, A^c = absorbance of control-test (correction factor), Vt = total volume of reagents in test tube and Vs = volume of serum

3.2.10Anthropometric measurements

Weight: This was measured with a mechanical weighing scale (SALTER 200, England) to the nearest 0.5kg, with the participants wearing light clothing and without shoes.

Height: Height was measured using a measuring tape to the nearest 0.1metre and the subjects were barefooted and in upright position.

Waist, hip and arm circumferences: These were measured to the nearest 0.1m, with the aid of a non-stretch measuring tape. The tape was snug around the body, but not pulled so tight to constrict.Waist circumference (WC) was measured at the midpoint between the lower ribs and the iliac crest. Hip circumference measurement was taken around the widest portion of the buttocks (WHO, 2011).Arm circumference was measured at the mid-point between the tip of the shoulder and the tip of the elbow, i.e., acromium and olecranon process (Devang *et al.*, 2016).

Body mass index (BMI) was obtained as weight (in kg) divided by square height (in m).

Calculation of ratios: Waist-to-hip and waist-to-height ratios were computed as the quotient between waist and hip circumferences and between WC and height (in m), respectively.

3.2.11 Blood pressure measurement:

This was carried out with the help of the hospital trained medical nurses using Mercury sphygmomanometer/Desk 605P (Suzuken Company, Japan.) with the subjects comfortably seated in an upright position.

3.2.12 Statistical Analysis

The data obtained were analyzed using students' T-test, one-way Analysis of Variance (ANOVA) and simple percentages with the aid of GraphPad Prism 5.3 (GraphPad Inc., USA). Values for $p \leq 0.05$ were considered statistically significant. Pearson's correlation was used to correlate between the various biochemical parameters.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Baseline Information of the Diabetic Subjects

The demographic and nutritional characteristics of the type 2 diabetic patients are presented in Table 4.1.Out of the 60 diabetic subjects investigated in this study, 10(16.7%), 18(30%) and 32(53.4%) were within the age intervals of 40-49, 50-59 and 60-70, respectively, with unequal prevalence between gender groups of ages 40-59. Majority of the subjects reside in urban areas 49 (81.7%), with greater number of these 28(46.7%) being males. Results showed that 55% of the subjects were gainfully employed, in which the main types of employment were: civil service (33.3%), trading (11.7%) and farming (10.0%).

Table 4.1: Demographic and nutritional characteristics of the type 2diabetes mellitus patients

VARIABLES	GROUPS	NUMBER OF PATIENTS (%)			
		ALL PATIENTS	Sex Grouping		
			Male	Female	p-value*
RESIDENCE	Urban	49 (81.7)	28 (46.7)	21 (35.0)	
	Rural	11 (18.3)	2(3.3)	9 (15.0)	0.0453**
EDUCATIONAL STATUS	No formal Education	14 (23.3)	0 (0.0%)	14 (23.3)	
	Primary Education	12 (20.0)	8 (13.3)	4 (6.7)	
	Secondary Education	15 (25.0)	12 (20.0)	3 (5.0)	
	Above Secondary Education	19 (31.7)	10 (16.7)	9 (15.0)	0.0001**
MARITAL STATUS	Single	2 (3.3)	2 (3.3)	0 (0.0)	
	Married	58 (96.7)	28 (46.7)	30 (50.0)	0.4915
AGE INTERVALS	40-49	10 (16.7)	4 (6.7)	6 (10.0)	
	50-59	18 (30.0)	10 (16.7)	8 (13.3)	
	60-70	32 (53.4)	16 (26.7)	16 (26.7)	0.6222
OCCUPATION	Employed Patients	33 (55.0)	18 (30.0)	15 (25.0)	
	Unemployed	27 (45.0)	12 (20.0)	15 (25.0)	0.6038

TYPE OF EMPLOYMENT	-Farmers	6 (10.0)	0 (0.0)	6 (10.0)	
	-Civil Servants	20 (33.3)	14 (23.3)	6 (10.0)	
	-Traders	7 (11.7)	5 (8.3)	2 (3.3)	0.0069**
DIET	Diabetic meals	31 (51.7)	15 (25.0)	16 (26.7)	
	Non diabetic meals	29 (48.3)	15 (25.0)	14 (23.3)	1.0000
INTAKE OF VEGETABLES	Daily	10 (16.7)	4 (6.7)	6 (10.0)	
	Three times a week	46 (76.7)	22 (36.7)	24 (40.0)	
	Once a week	4 (6.7)	4 (6.7)	0 (0.0)	0.1061

*Chi-square / Fisher's exact analysis of male and female observations. **Significant observations. Values in brackets represent the percentage (%) of thetype 2 diabetic patients.

The clinical characteristics of the type 2 diabetic patients are presented in Table 4.2. In this study,majority of the patients (56.6%) were diagnosed / started treatment for diabetes in the last 2-10 years, 33.3% of which were female. Out of a total of 44 (73.3 %) T2DM patients that have family history of the disease, 43.3% were males while 30% were females. Only about 28.3% of the T2DM patients had hypertension in association with T2DM, majority of these participants were male T2DM patients (18.3%). Greater percentages of the T2DM patients that exhibited systolic BP $\geq$130 mmHg and diastolic BP $\geq$ 90 mmHg were females.

Table 4.2: Clinical characteristics of the type 2 diabetes mellitus patients

VARIABLES	GROUPS	NUMBER OF PATIENTS (%)			
		ALL PATIENTS	Sex Grouping		
			Male	Female	p-value*
DIAGNOSIS/TREATMENT DURATION	≤ 1 year	17 (28.4)	10 (16.7)	7 (11.7)	
	2-10 years	34 (56.6)	14 (23.3)	20 (33.3)	
	> 10 years	9 (15.0)	6 (10.0)	3 (5.0)	0.2741
CO-MORBIDITY (WITH HYPERTENSION)	Present	17 (28.3)	11 (18.3)	6 (10.0)	
	Absent	43 (71.7)	19 (31.7)	24 (40.0)	0.2516
THOSE ON DRUG(S) OTHER THAN DIABETES DRUGS	Antibiotics only	3 (5.0)	3 (5.0)	0 (0.0)	
	Antibiotics &hypertensive drugs	4 (6.7)	4 (6.7)	0 (0.0)	
ADDITIONAL SYMPTOMS OVER TIME	Feet numbness/leg pain	26 (43.3)	15 (25.0)	11 (18.3)	
	Chest pain	11 (18.4)	10 (16.7)	1 (1.7)	
	Increased weakness	13 (21.7)	6 (10.0)	7 (11.7)	0.0642
FAMILY HISTORY OF DIABETES	Present	44 (73.3)	26 (43.3)	18 (30.0)	
	Absent	16 (26.7)	4 (6.7)	12 (20.0)	0.0391**
REGULAR FASTING BLOOD SUGAR TEST	≤ 3 times a week	12 (20.0)	7 (11.7)	5 (8.3)	
	Once in 2 weeks	42 (70.0)	19 (31.7)	23 (38.3)	
	Once a month	6 (10.0)	4 (6.7)	2 (3.3)	0.5013
BLOOD GLUCOSE LEVEL	Fluctuates	58 (96.7)	28 (46.7)	30 (50.0)	
	Decreased	2 (3.3)	2 (3.3)	0 (0.0)	0.4915
BLOOD PRESSURE	Systolic <130 mmHg	35 (58.3)	21 (35.0)	14 (23.3)	
	≥130 mmHg	25 (41.7)	9 (15.0)	16 (26.7)	
	≥140mmHg	19 (31.6)	5 (8.3)	14 (23.3)	0.0354**
	Diastolic <90 mmHg	52 (86.7)	28 (46.7)	24 (40.0)	
	≥90 mmHg	8 (13.3)	2 (3.3)	6 (10)	0.2542

*Chi-square / Fisher's exact analysis of male and female observations. **Significant observations. Values in brackets represent the percentage (%) of the type 2 diabetic patients.

4.1.2 Fasting blood glucose

Fasting blood glucose concentration increased significantly in the diabetic subjects (176.42 ± 46.86mg/dl) compared to the control (93.87 ± 15.33 mg/dl).The same trend was observed between groups of both the male and female subjects.

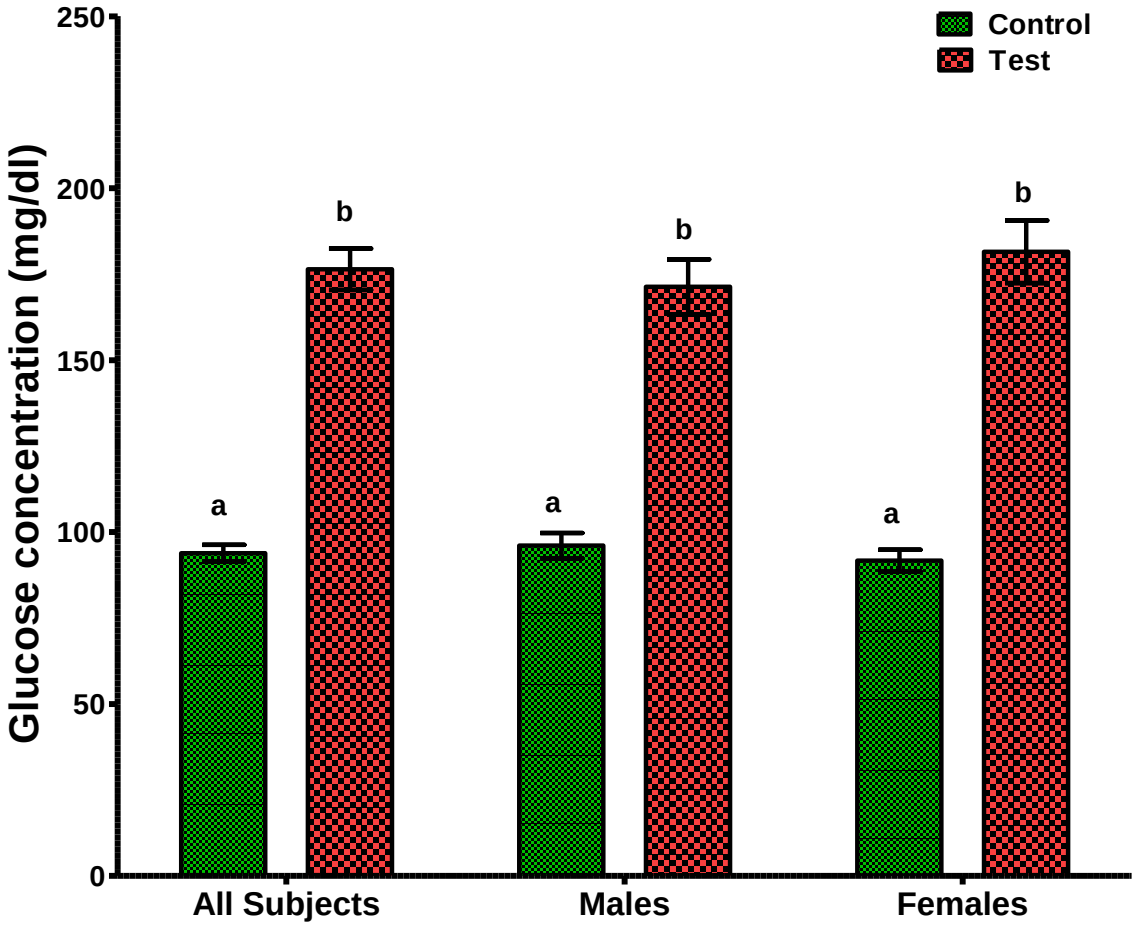

Figure 4.1: Blood glucose concentration (mg/dl) of diabetic and non-diabetic subjects. Data are presented as mean ± standard deviation. Bars bearing different letters are statistically significant ($p<0.05$) within and between groups.

4.1.3 Lipid profile results

i. Total cholesterol

Significant increase in total cholesterol concentration was observed in diabetics (5.68 ± 0.66mmol/L) compared with the non-diabetic subjects (3.55 ± 0.89 mmol/L).The same trend was observed for the male and female subjects. However, the male control showed significantly ($p<0.05$) lower total cholesterol concentration than female control.

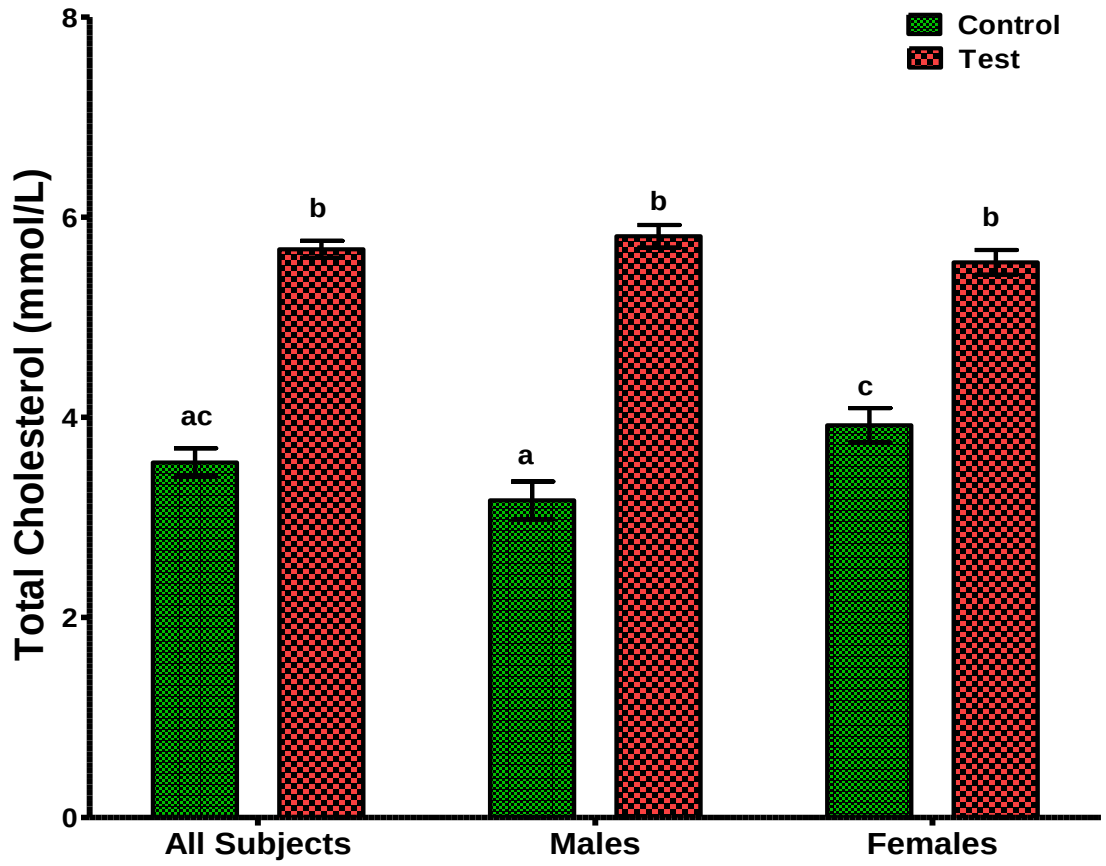

Figure 4.2: Total cholesterol concentration (mmol/L) of diabetic and non-diabetic subjects. Data are presented as mean ± standard deviations. Bars bearing different letters are statistically significant ($p<0.05$) within and between groups.

ii. High density lipoprotein cholesterol

HDL-c concentration was significantly ($p<0.05$) lower in the diabetic subjects (1.20 ± 0.40 mmol/L) than the non-diabetics (1.61 ± 0.26 mmol/L), with the male diabetics having significantly ($p<0.05$) lower HDL-c than the female diabetics.

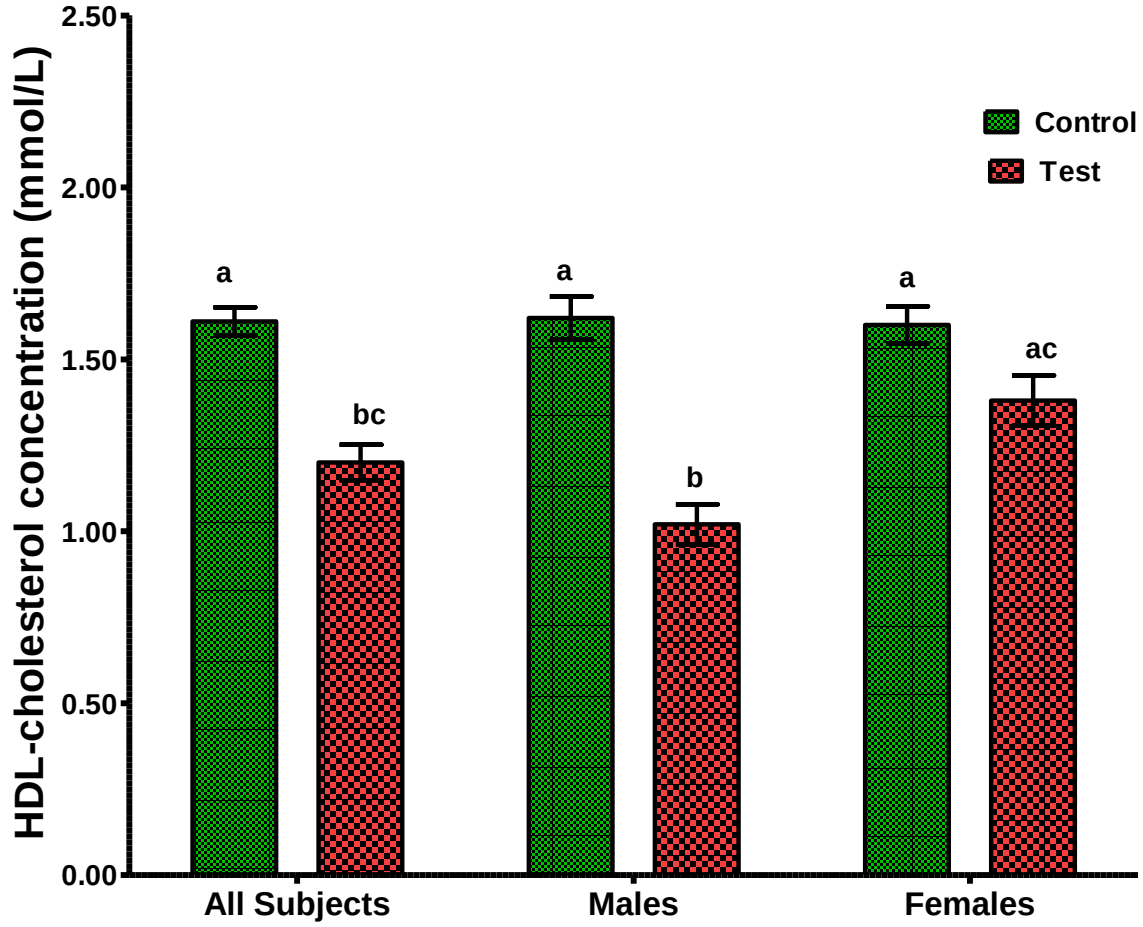

Figure 4.3: HDL-cholesterol concentration (mmol/L) of diabetic and non-diabetic subjects. Data are presented as mean ± standard deviations. Bars bearing different letters are statistically significant ($p<0.05$)within and between groups

iii. Triacylglycerol

The diabetic subjects had significantly ($p<0.05$) higher triacylglycerol concentration (2.63 ± 0.71 mmol/L)in comparison with the non-diabetic subjects(1.03 ± 0.40 mmol/L). Similar trend was observed among the male and female subjects.

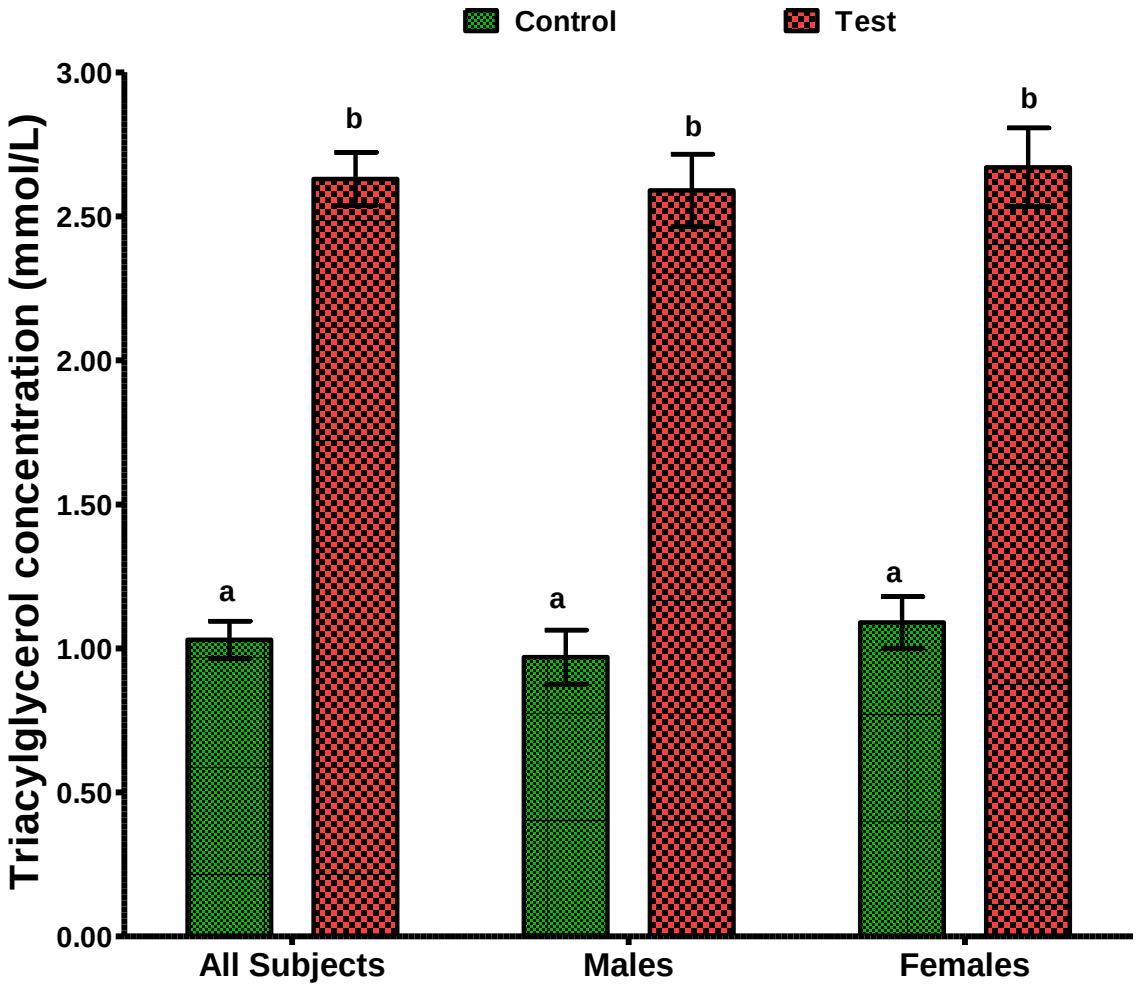

Figure 4.4: Triacylglycerol concentration (mmol/L) of diabetic and non-diabetic subjects. Data are presented as mean ± standard deviations. Bars bearing different letters are statistically significant ($p<0.05$)within and between groups.

iv. Very low density lipoprotein

VLDL-c concentration was significantly ($p<0.05$) higher in diabetics (1.20 ± 0.32 mmol/L) compared to non-diabetic subjects (0.47 ± 0.19 mmol/L).This was also observed among the male and female subjects.

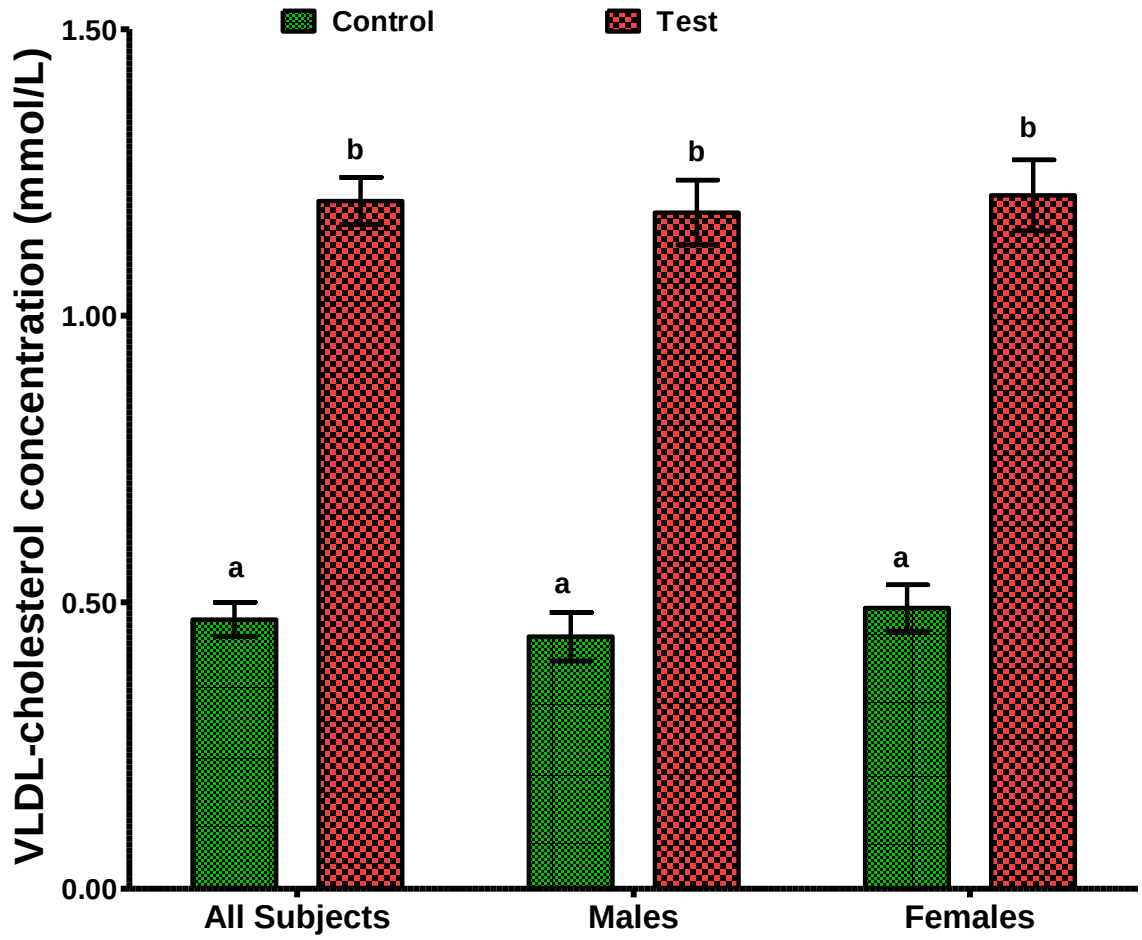

Figure 4.5: VLDL-cholesterol concentration (mmol/L) of diabetic and non-diabetic subjects. Data are presented as mean ± standard deviations. Bars bearing different letters are statistically significant ($p<0.05$)within and between groups.

v. Low density lipoprotein cholesterol

The results of this study showed that LDL-c concentrations increased significantly in diabetic subjects (5.68 ± 0.74 mmol/L) compared to non-diabetic subjects (2.40 ± 0.82 mmol/L). Similar trend was observed among the male and female subjects.

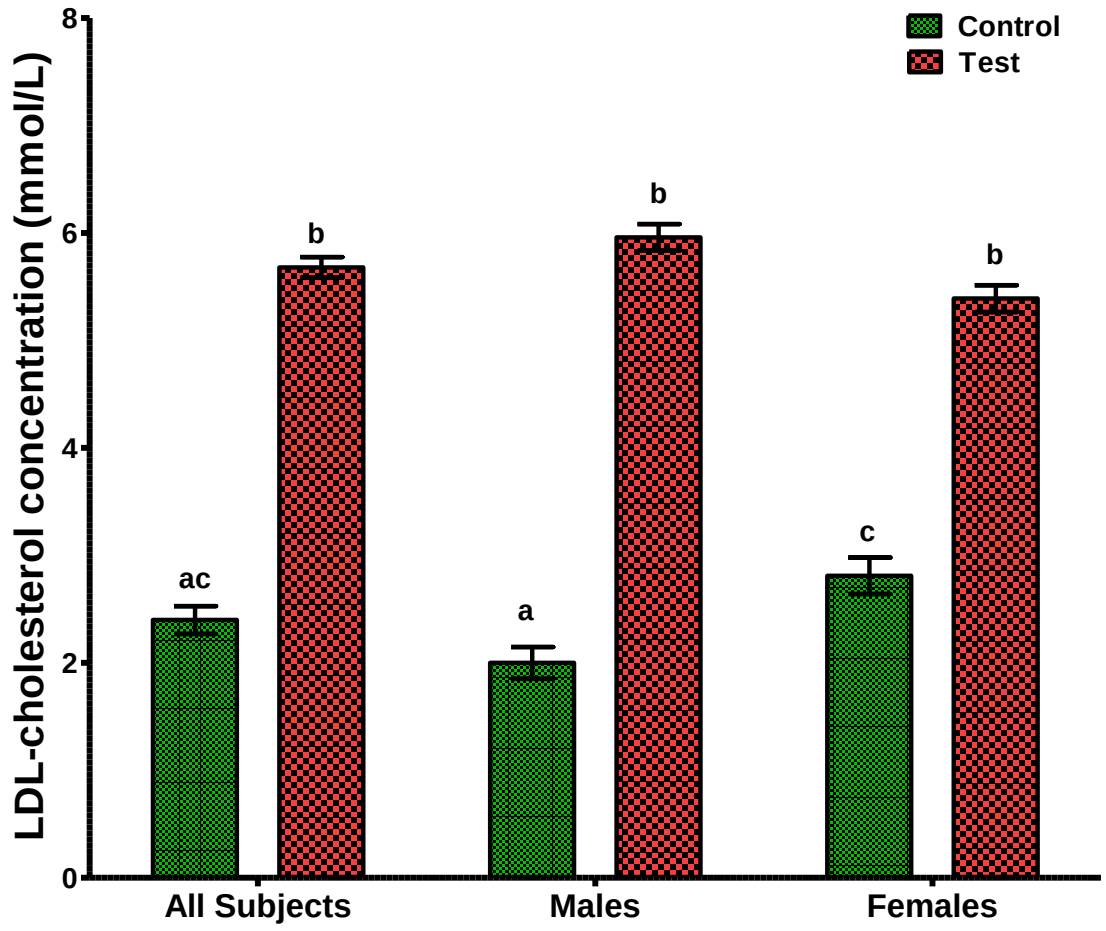

Figure 4.6: LDL-cholesterol concentration (mmol/L) of diabetic and non-diabetic subjects. Data are presented as mean ± standard deviations. Bars bearing different letters are statistically significant ($p<0.05$) within and between groups.

4.1.4Atherogenic Indices

i.Non-HDL cholesterol

nHDL-c concentration increased significantly in diabetics (4.48 ± 0.79 mmol/L) than non-diabetics (1.95 ± 0.83 mmol/L), with a slightly significant ($p<0.05$) increase in males than female diabetics.

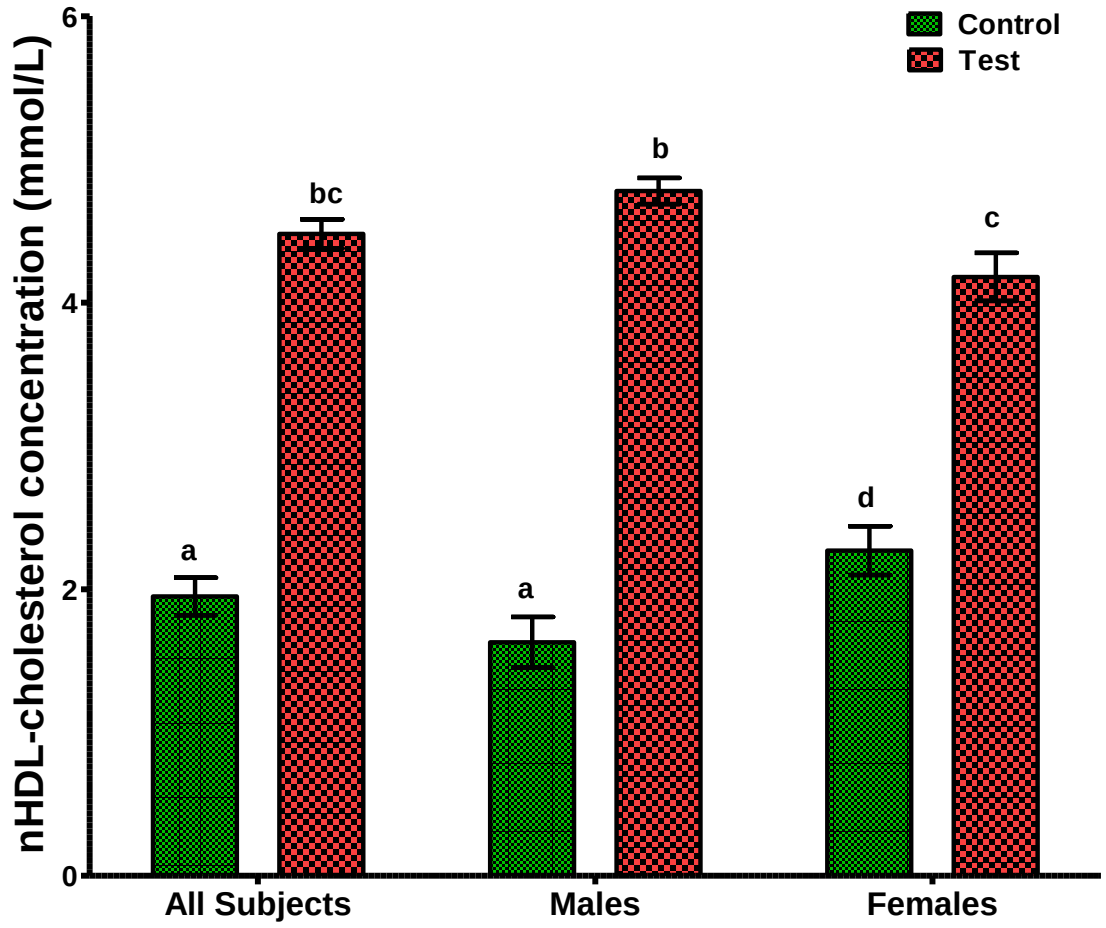

Figure 4.7: Non-HDL-cholesterol concentration (mmol/L) of diabetic and non-diabetic subjects. Data are presented as mean ± standard deviations. Bars bearing different letters are statistically significant ($p<0.05$) within and between groups.

ii. Castelli's risk index1

A significant ($p<0.05$)increase in TC/HDL-c ratio (CRI-1) was observed in diabetics (5.31 ± 1.19) when compared to non-diabetics (2.27 ± 0.70), and also in males compared to female diabetics.

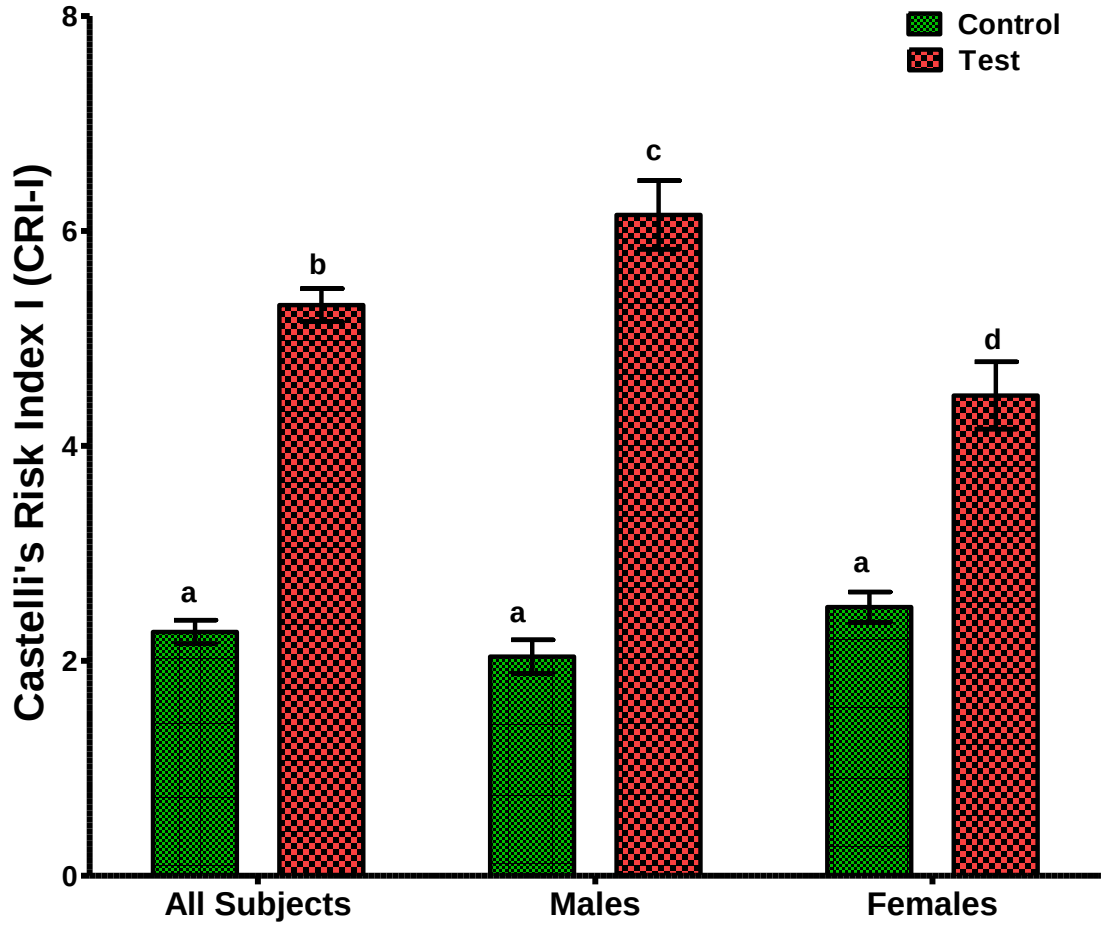

Figure 4.8: Castelli's risk index I (CRI-I) of diabetic and non-diabetic subjects. Data are presented as mean ± standard deviation. Bars bearing different letters are statistically significant ($p<0.05$) within and between groups.

iii. Castelli's risk index II

A significant increase in LDL-c /HDL-c ratio (CRI-II) was observed in diabetics (5.29 ± 2.07) in comparison with non-diabetics (1.55 ± 0.63), and also in diabetic males compared to their female counterparts.

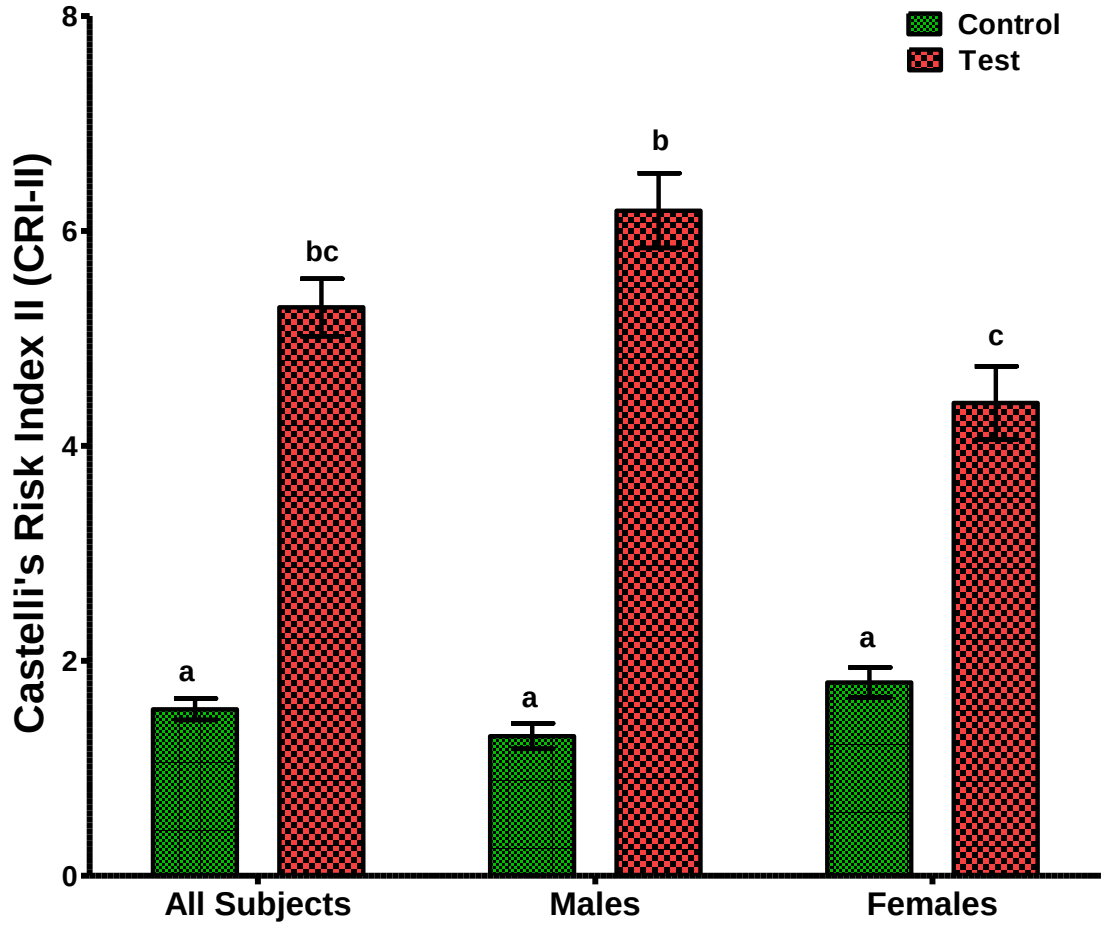

Figure 4.9: Castelli's risk index II (CRI-II) of diabetic and non-diabetic subjects. Data are presented as mean ± standard deviations. Bars bearing different letters are statistically significant ($p<0.05$) within and between groups.

iv. Atherogenic coefficient (AC)

The ratio of nHDL-c to HDL-c (atherogenic coefficient) increased significantly ($p<0.05$) in diabetics (4.31 ± 1.91) compared to the non-diabetics (1.27 ± 0.63).The AC was also significantly ($p<0.05$) higher in males than female diabetics.

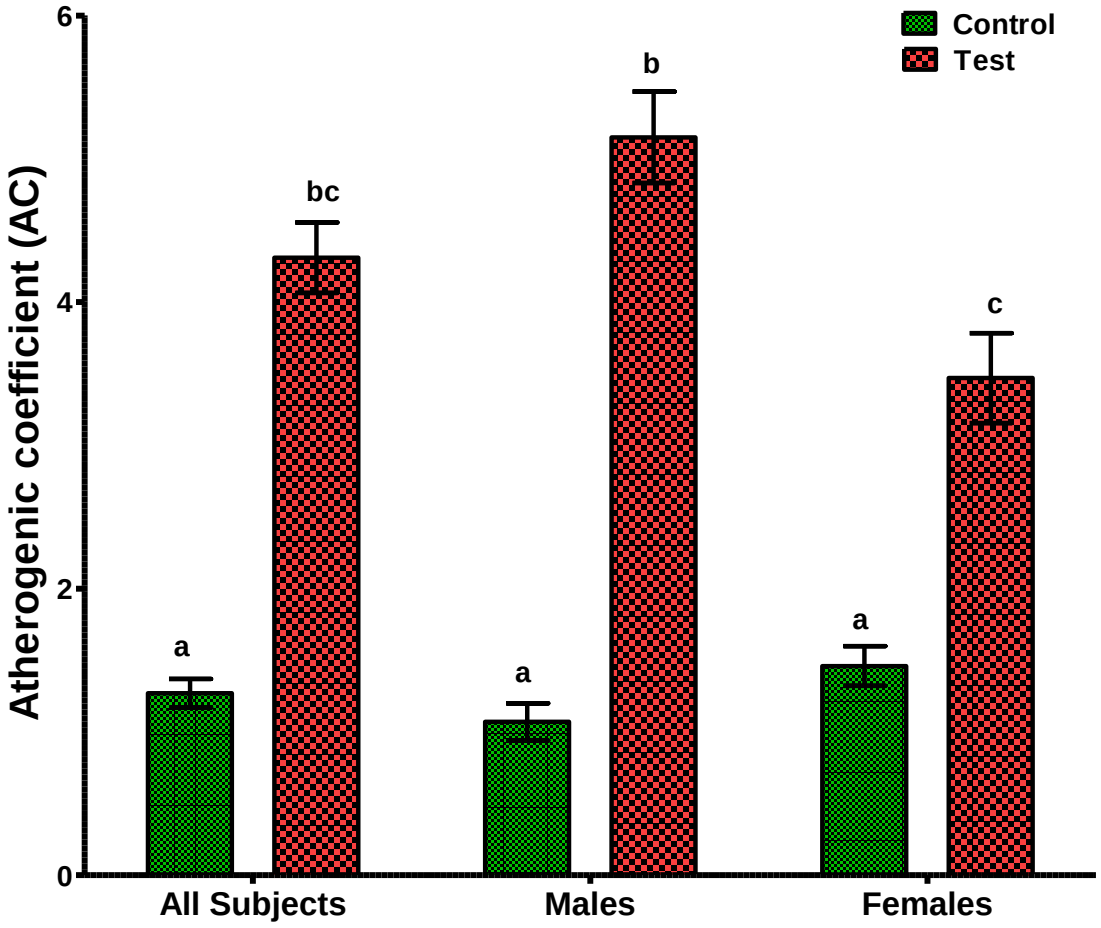

Figure 4.10: Atherogenic coefficient of diabetic and non-diabetic subjects. Data are presented as mean ± standard deviations. Bars bearing different letters are statistically significant ($p<0.05$) within and between groups.

v. Atherogenic index of plasma

There was a significant ($p<0.05$) increase in the atherogenic index of plasma (AIP) of diabetics (0.33 ± 0.11) compared to the non-diabetics (-0.21 ± 0.16).The AIP magnitude was generally positive amongst the diabetics but negative in the control groups.

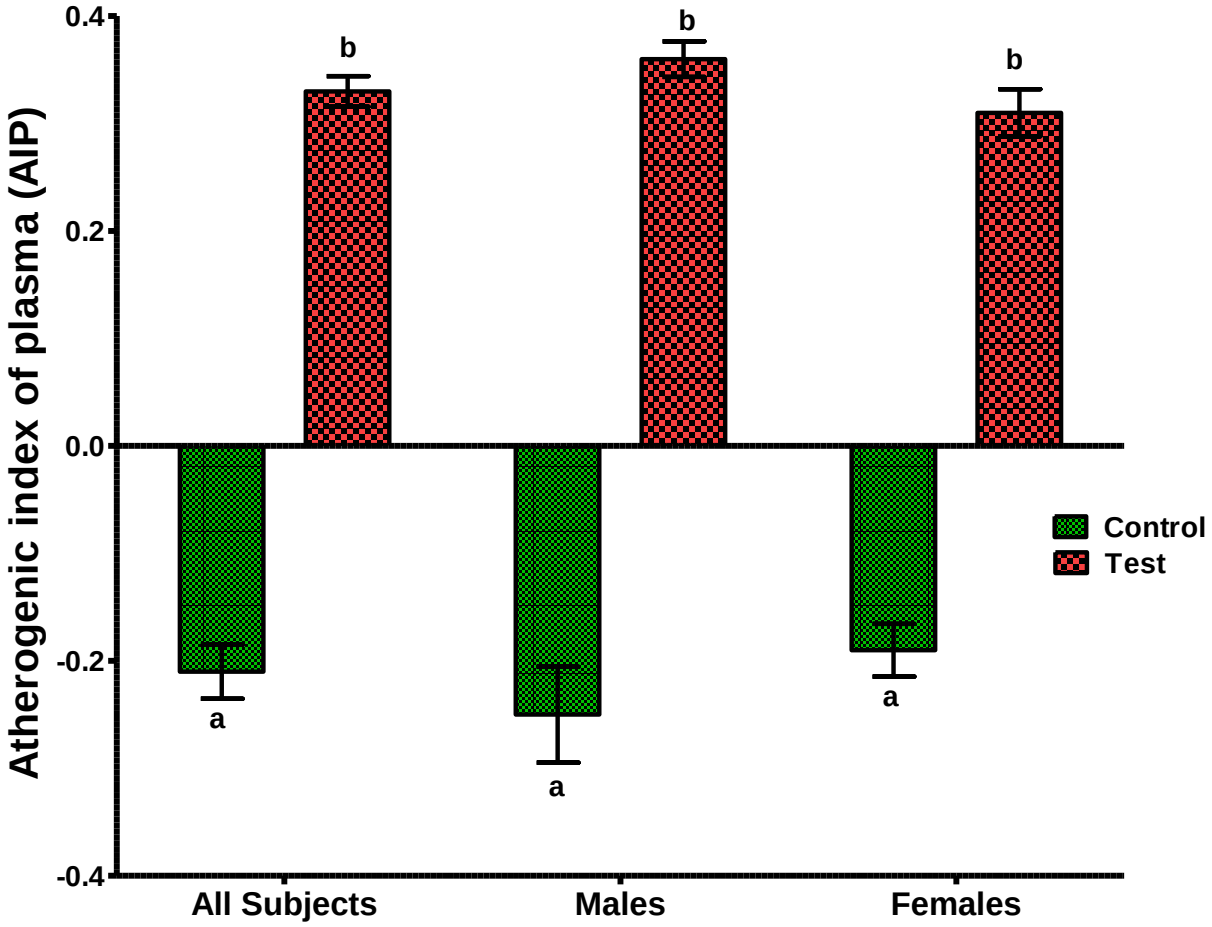

Figure 4.11: Atherogenic index of plasma (AIP) of diabetic and non-diabetic subjects. Data are presented as mean ± standard deviations. Bars bearing different letters are statistically significant ($p<0.05$) within and between groups.

4.1.5 Oxidative stress parameters

i. Reduced glutathione concentration

GSH concentration decreased significantly ($p<0.05$)in diabetic subjects (3.05 ± 0.78µmol/L) in comparison with the non-diabetics (4.40 ± 0.88 µmol/L).

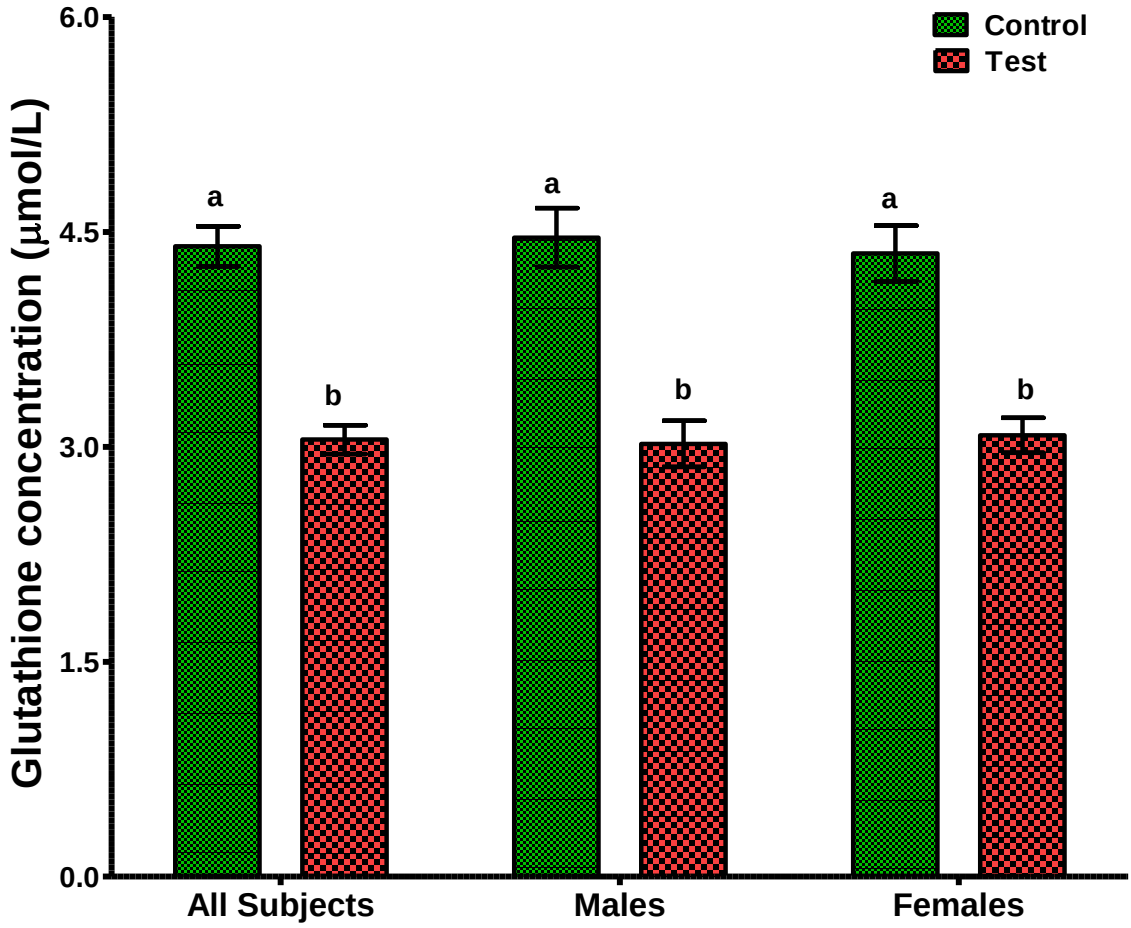

Figure 4.12: Reduced glutathione concentration (µmol/L) of diabetic and non-diabetic subjects. Data are presented as mean ± standard deviations. Bars bearing different letters are statistically significant ($p<0.05$)within and between groups.

ii. Superoxide dismutase activity

SOD activity significantly ($p<0.05$)decreased in diabetics (12.85 ± 2.16U/ml) than non-diabetic subjects (18.94 ± 1.47 U/ml). Similar trend was observed among the sex groupings.

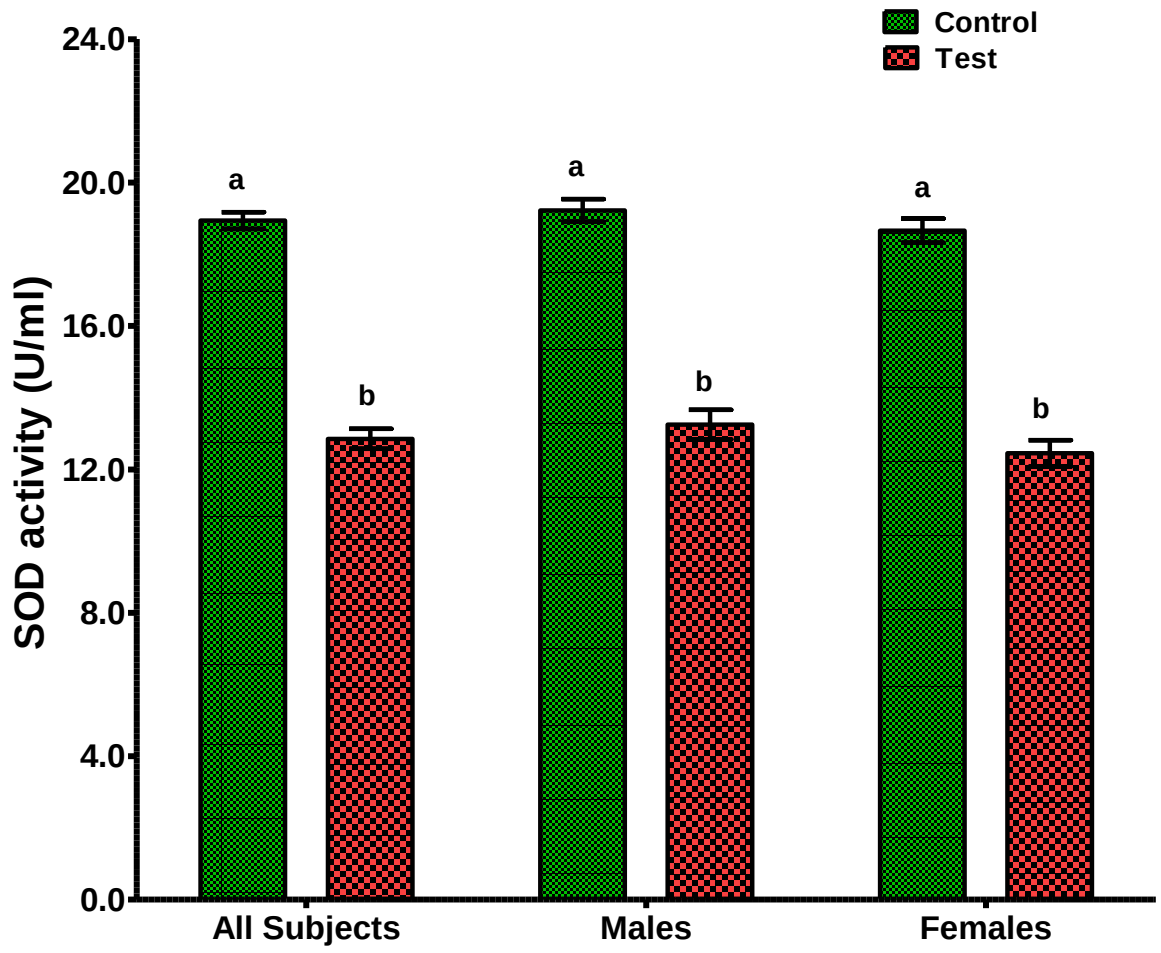

Figure 4.13: Superoxide dismutase activity (U/ml) of diabetic and non-diabetic subjects. Data are presented as mean ± standard deviations. Bars bearing different letters are statistically significant ($p<0.05$) within and between groups.

iii. Malondialdehyde concentration

MDA concentration was significantly ($p<0.05$)higher in diabetic subjects (2.75 ± 0.58nmol/ml) in comparison with the non-diabetics (1.36 ± 0.26 nmol/ml).

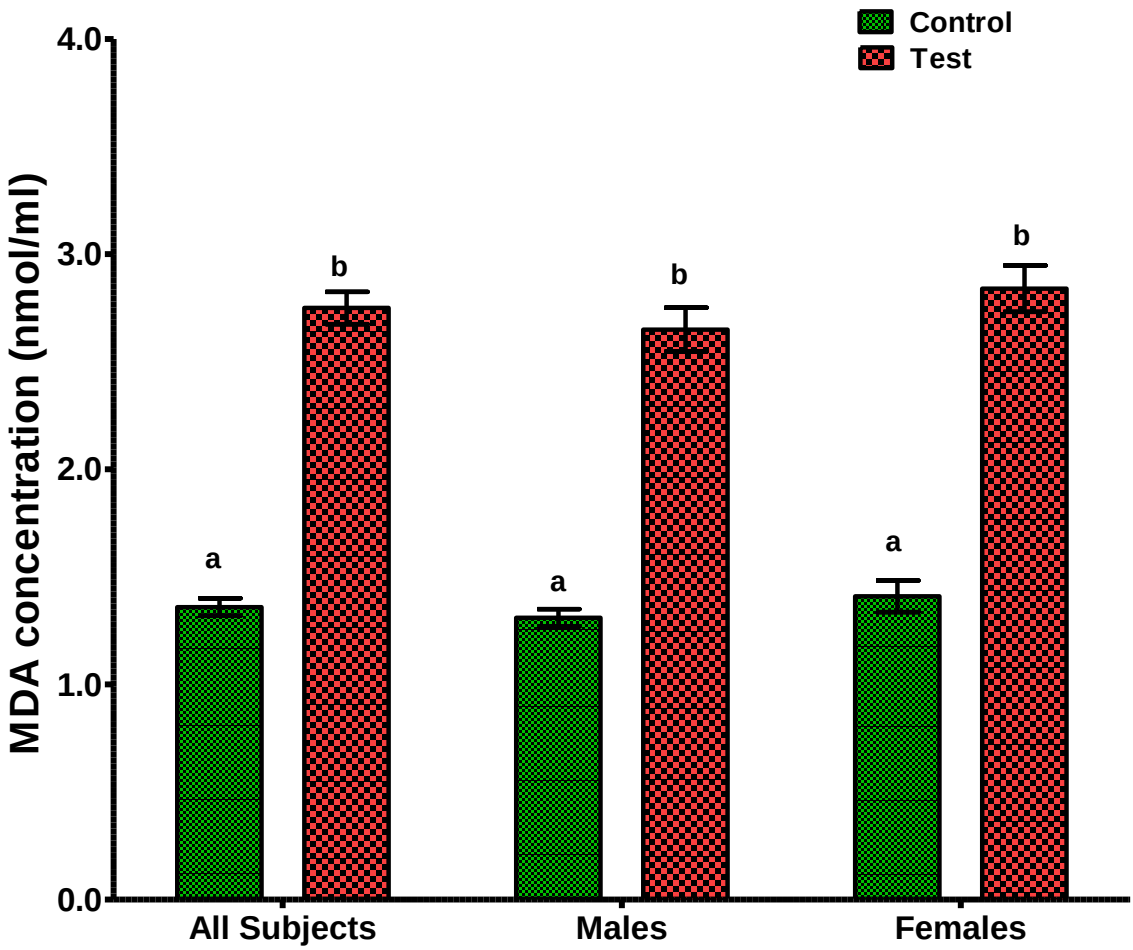

Figure 4.14: Malondialdehyde (MDA) concentration (nmol/ml) of diabetic and non-diabetic subjects. Data are presented as mean ± standard deviations. Bars bearing different letters are statistically significant ($p<0.05$) within and between groups.

iv. Glutathione peroxidase activity

GPx activity significantly ($p<0.05$)decreased in diabetic subjects (0.58 ± 0.06U/ml) than non-diabetic subjects (0.78 ± 0.12U/ml).

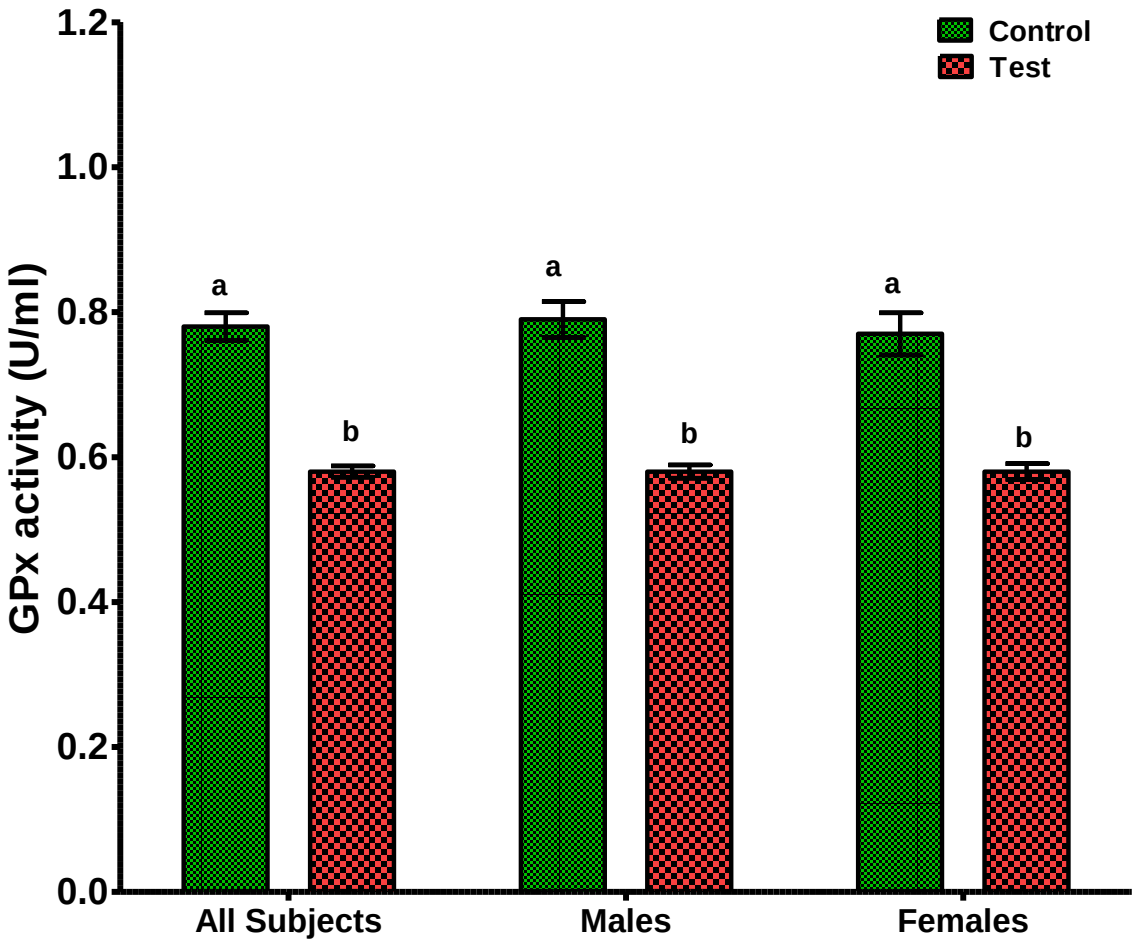

Figure 4.15: Glutathione peroxidase activity (U/ml) of diabetic and non-diabetic subjects. Data are presented as mean ± standard deviations. Bars bearing different letters are statistically significant ($p<0.05$) within and between groups.

v. Catalase activity

CAT activity significantly ($p<0.05$) decreased in diabetic subjects (40.97 ± 7.50kU/l) compared to non-diabetic subjects (58.52 ± 8.68kU/l).

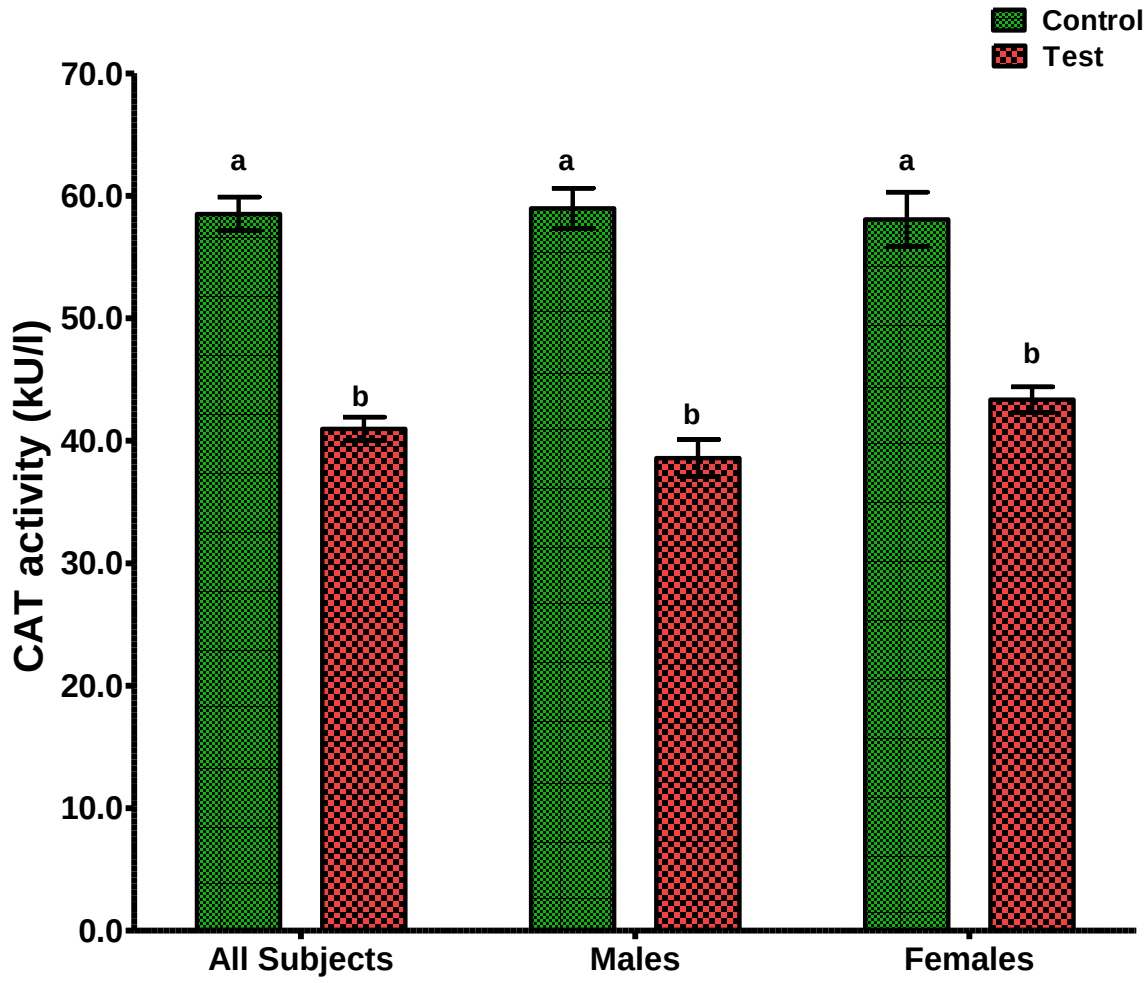

Figure 4.16: Catalase activity (kU/l) of diabetic and non-diabetic subjects. Data are presented as mean ± standard deviations. Bars bearing different letters are statistically significant ($p<0.05$) within and between groups.

4.1.6 Anthropometric Indices

i. Body mass index

From the results of this study, a significantly ($p<0.05$)higher BMI was observed among diabetics (27.43 ± 5.64 kg/m^2) compared to non-diabetics (20.98 ± 2.92 kg/m^2), and also in females than male subjects.

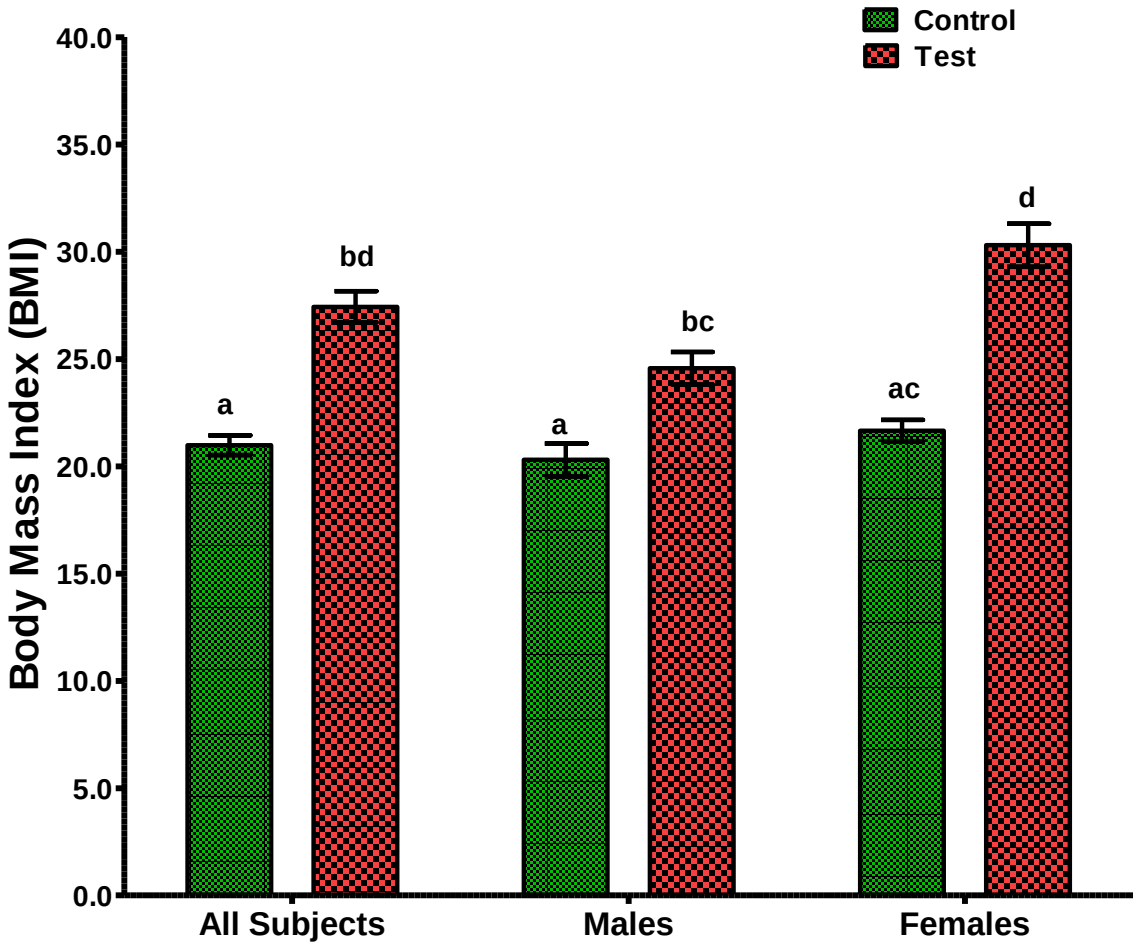

Figure 4.17: Body mass index (BMI) (kg/m2) of diabetic and non-diabetic subjects. Data are presented as mean ± standard deviations. Bars bearing different letters are statistically significant ($p<0.05$) within and between groups.

ii. Waist circumference

The waist circumference (WC) was significantly ($p<0.05$)higher in diabetics (0.99 ± 0.08m) than non-diabetics (0.84 ± 0.07m), and also in females than male subjects.

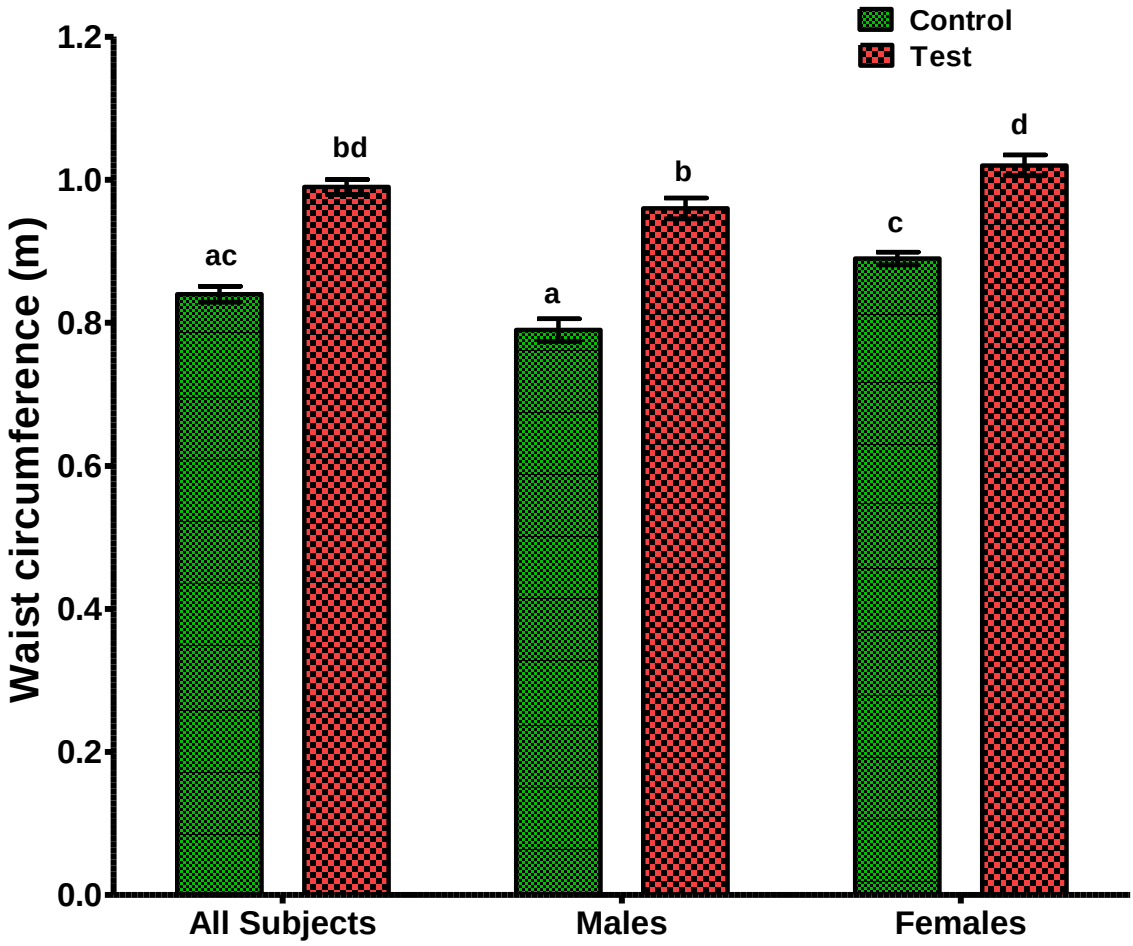

Figure 4.18: Waist circumference (m) of diabetic and non-diabetic subjects. Data are presented as mean ± standard deviations. Bars bearing different letters are statistically significant ($p<0.05$) within and between groups.

iii. **Hip circumference**

Slight but non-significant ($p>0.05$) increase was noted in the hip circumference (HC) of the diabetics (1.02 ± 0.10m) compared to the non-diabetics (1.00 ± 0.10m), and also among the females than male diabetic subjects.

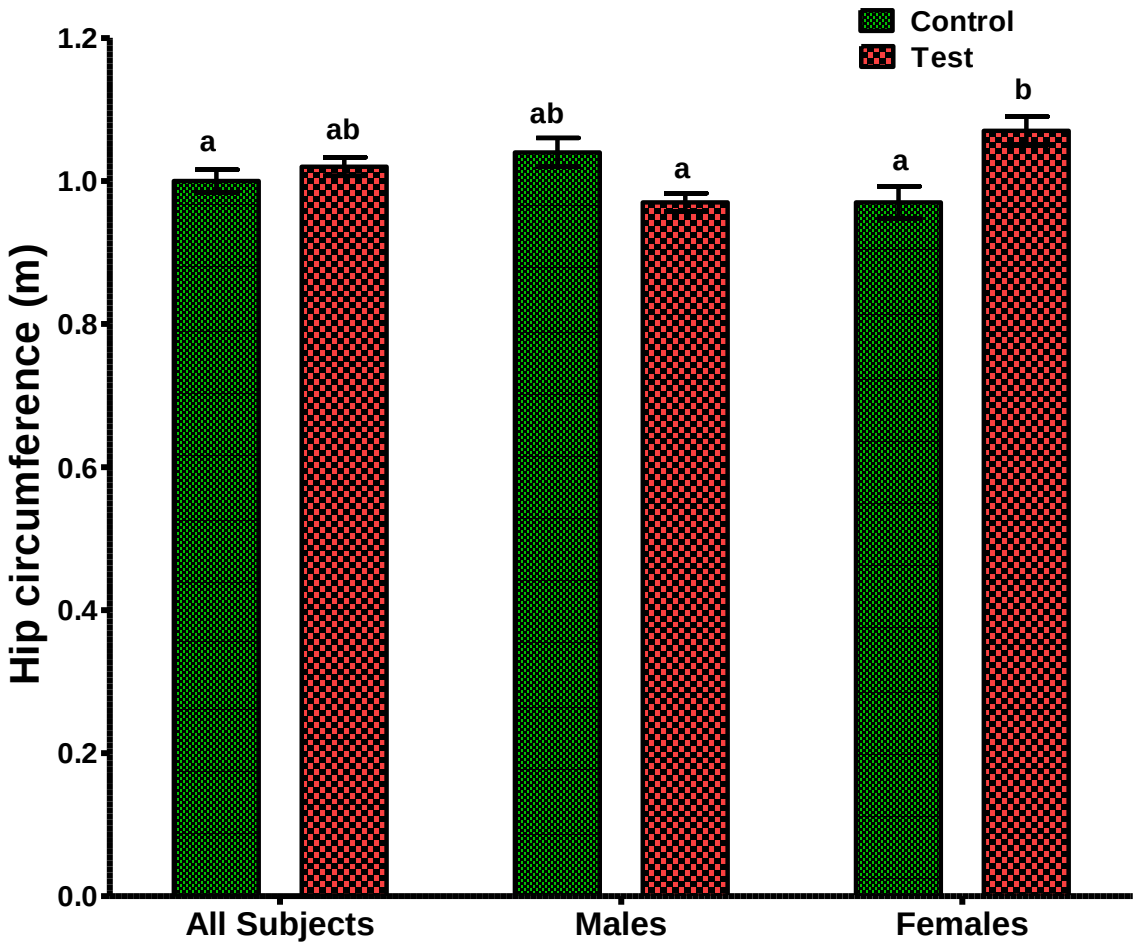

Figure 4.19: Hip circumference (m) of diabetic and non-diabetic subjects. Data are presented as mean ± standard deviations. Bars bearing different letters are statistically significant ($p<0.05$) within and between groups.

iv. Waist to hip ratio

The WHR was significantly ($p<0.05$) higher in diabetic subjects (0.98 ± 0.09) than non-diabetics (0.84 ± 0.10). Similar results were observed in both the male and female subjects.

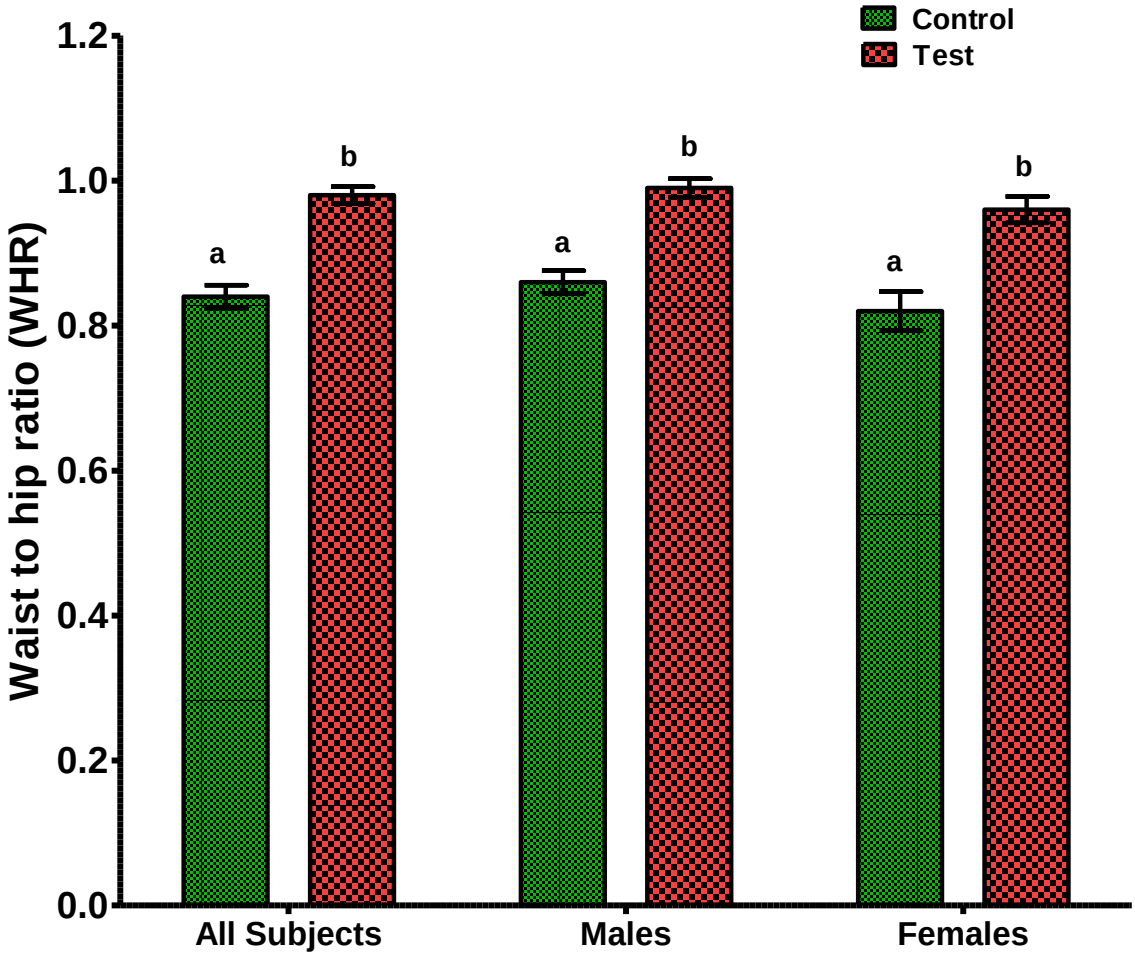

Figure 4.20: Waist to hip ratio (WHR) of diabetic and non-diabetic subjects. Data are presented as mean ± standard deviations. Bars bearing different letters are statistically significant ($p<0.05$) within and between groups.

v. Waist to height ratio

A significant ($p<0.05$) increase in WHtR was observed in diabetics (0.61 ± 0.07) than the controls (0.50 ± 0.04), and also in female than male diabetic subjects.

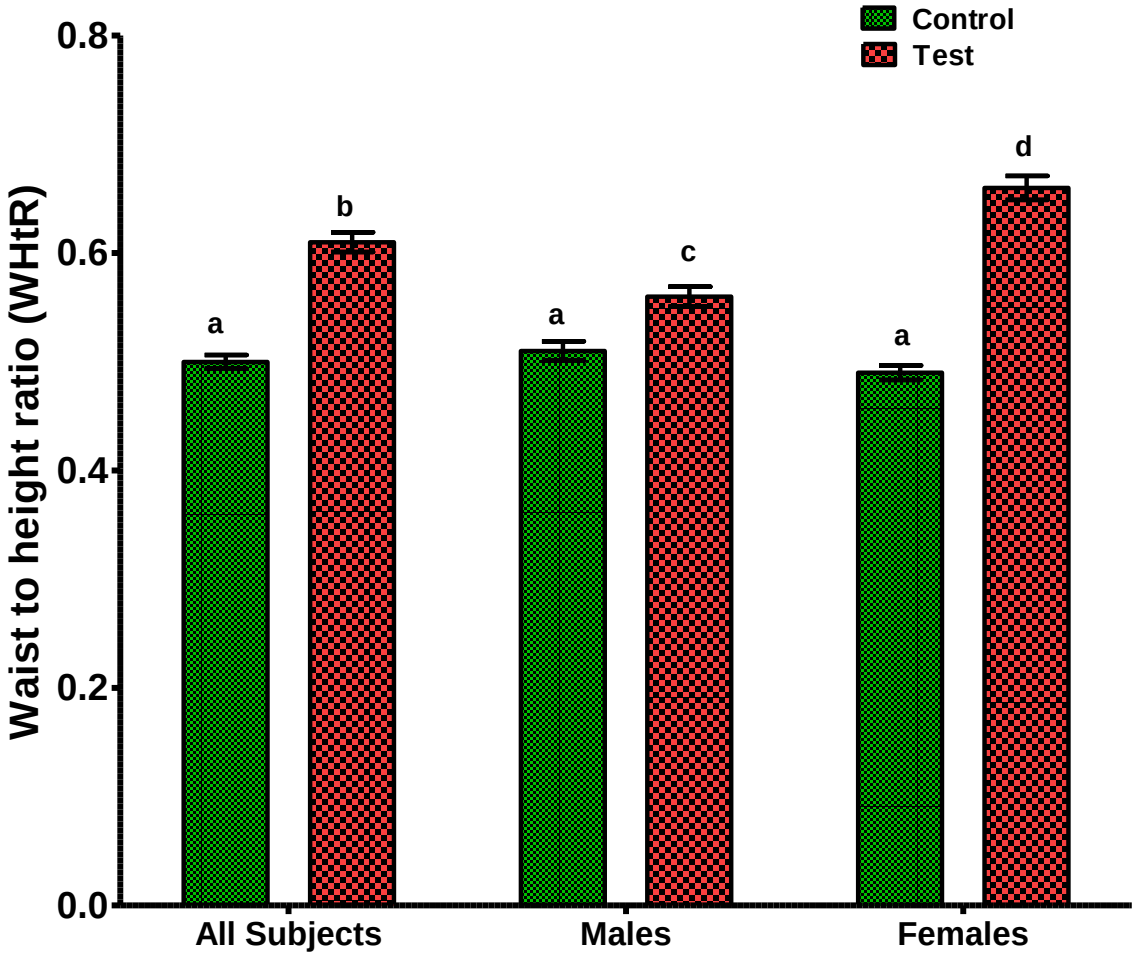

Figure 4.21: Waist to height ratio (WHtR) of diabetic and non-diabetic subjects. Data are presented as mean ± standard deviations. Bars bearing different letters are statistically significant ($p<0.05$) within and between groups.

vi. Arm circumference

There was a significant ($p<0.05$) increase in the arm circumference (AC) of the diabetics (0.35 ± 0.03 m) compared to the non-diabetics (0.31 ± 0.04 m).The same trend was observed within the groups, but no significant ($p<0.05$) difference was seen between male and female diabetic subjects.

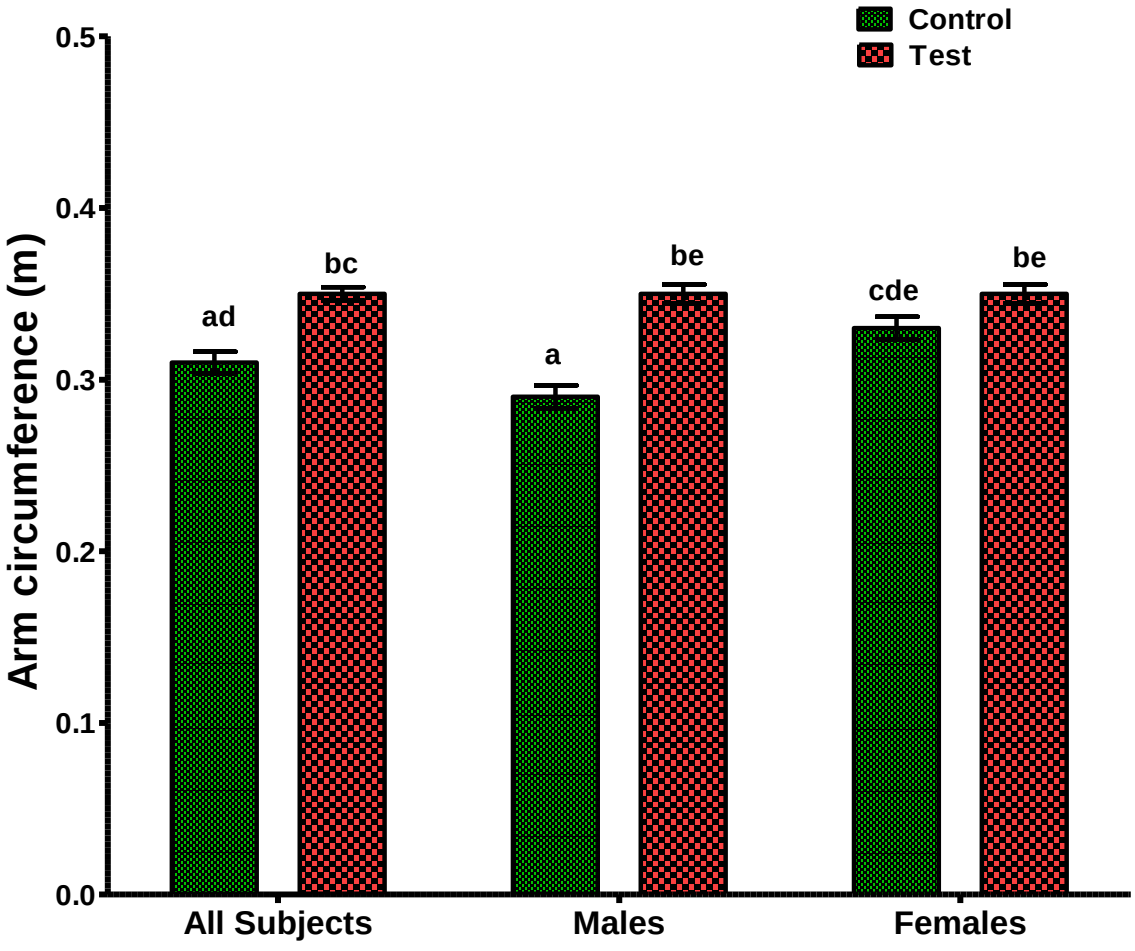

Figure 4.22: Arm circumference (m) of diabetic and non-diabetic subjects. Data are presented as mean ± standard deviations. Bars bearing different letters are statistically significant ($p<0.05$) within and between groups.

4.1.7 Blood Pressure

i. Systolic blood pressure

Diabetic subjects showed a significant ($p<0.05$) increase in systolic blood pressure (140.92 ± 11.81mmHg) when compared to non-diabetics (125.50 ± 14.22 mmHg). Female diabetics showed increase in systolic blood pressure than male diabetics.

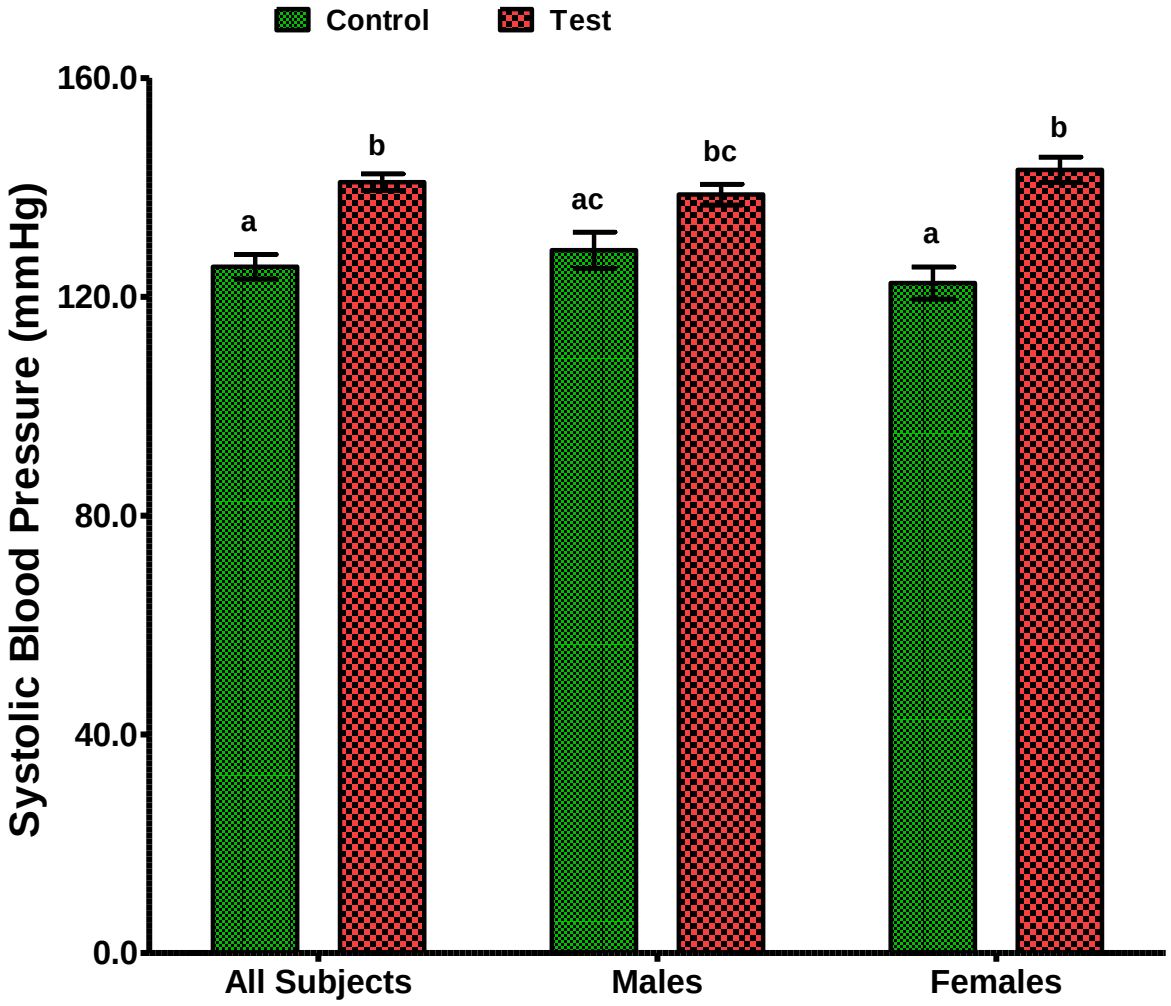

Figure 4.23: Systolic blood pressure (mmHg) of diabetic and non-diabetic subjects. Data are presented as mean ± standard deviations. Bars bearing different letters are statistically significant ($p<0.05$) within and between groups.

ii. Diastolic blood pressure

A significant ($p<0.05$) increase was noted in diastolic blood pressure of diabetic subjects (82.33 ± 6.67 mmHg) compared to control subjects (78.50 ± 8.34 mmHg), and also in female than male diabetics.

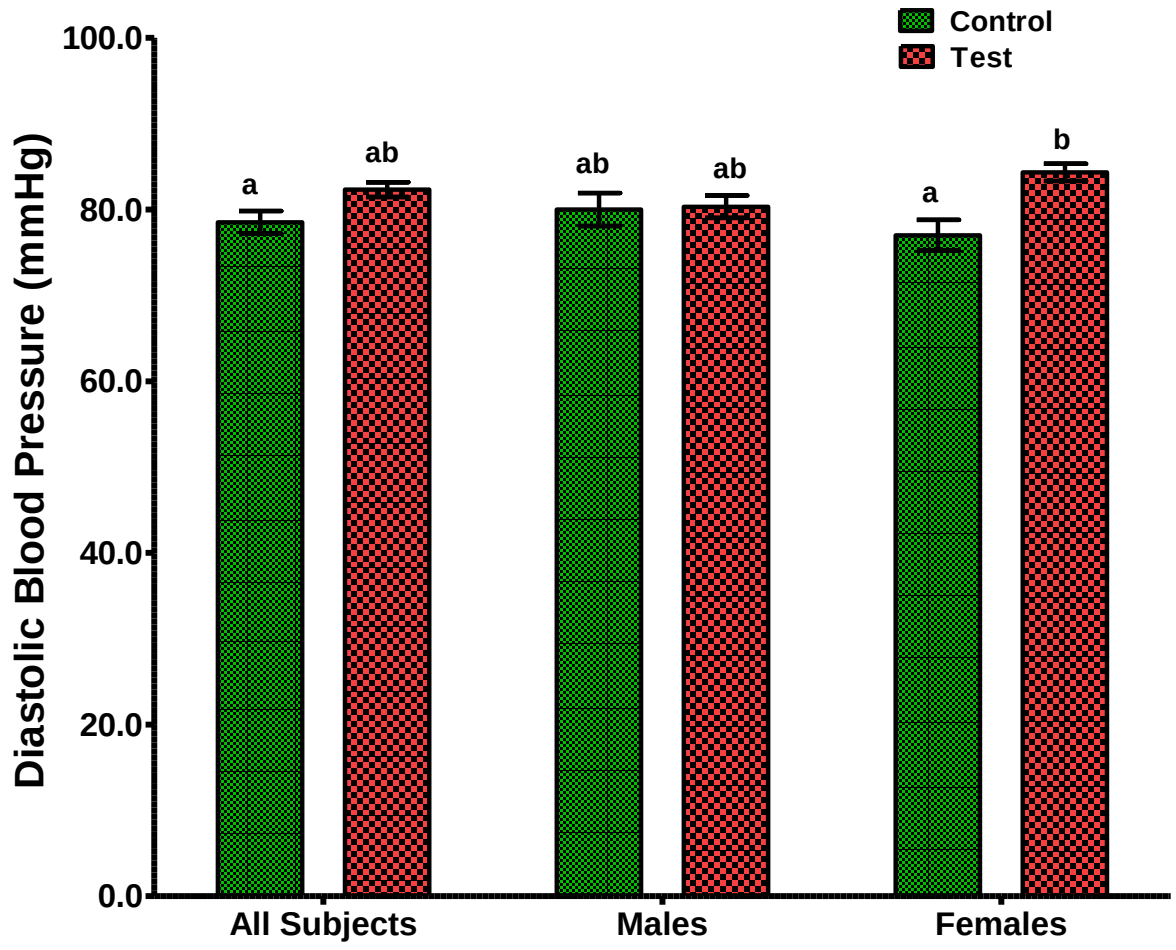

Figure 4.24: Diastolic blood pressure (mmHg) of diabetic and non-diabetic subjects. Data are presented as mean ± standard deviations. Bars bearing different letters are statistically significant ($p<0.05$) within and between groups.

4.1.8 Correlation studies

Fasting blood glucose (FBG) concentration of the diabetic subjects was significantly ($p<0.05$) and positively correlated with their MDA (Malondialdehyde) concentration but negatively related to their GSH (Glutathione) concentration. MDA was also positively related to SOD (Superoxide dismutase) activity of the diabetic subjects (Table 4.3)

Table 4.3: Correlation coefficient (p value) matrix of blood glucose concentration and oxidative stress parameters of diabetic subjects (n = 60).

	FBG (mg/dl)	GSH (µmol/L).	GPx (U/ml)	MDA (nmol/ml)	SOD (U/ml)	CAT (kU/l)
FBG		-0.27 (0.038)*	0.01 (0.934)	0.30 (0.019)*	0.01 (0.918)	-0.08 (0.549)
GSH	-0.27 (0.038)*		-0.20 (0.129)	-0.19 (0.137)	-0.04 (0.734)	0.22 (0.099)
GPx	0.01 (0.934)	-0.20 (0.129)		-0.21 (0.107)	0.08 (0.557)	-0.15 (0.250)
MDA	0.30 (0.019)*	-0.19 (0.137)	-0.21 (0.107)		0.40 (0.001)*	0.02 (0.866)
SOD	0.01 (0.918)	-0.04 (0.734)	0.08 (0.557)	0.40 (0.001)*		-0.22 (0.094)
CAT	-0.08 (0.549)	0.22 (0.099)	-0.15 (0.250)	0.02 (0.866)	-0.22 (0.094)	

*Statistically significant ($p<0.05$).The respective p values are in the brackets.

The fasting blood glucose concentration of the diabetic subjects was negatively correlated with TG (Triacylglycerol) and VLDL-c (Very low density lipoprotein cholesterol) concentrations (Table 4.4). On the other hand, LDL-c (low density lipoprotein cholesterol) was positively related to TC (Total cholesterol) but negatively correlated with HDL-c (High density lipoprotein cholesterol) of the subjects.

Table 4.4: Correlation coefficient (p value) matrix of blood glucose concentration and lipid profile parameters of diabetic subjects (n = 60).

	FBG(mg/dl)	TC (mmol/L)	TG (mmol/L)	HDL-c (mmol/L)	VLDL-c (mmol/L)	LDL-c (mmol/L)
TC	0.20 (0.122)		-0.23 (0.077)	-0.08 (0.546)	-0.23 (0.077)	0.83 (0.000)*
TG	-0.30 (0.019)*	-0.23 (0.077)		0.36 (0.004)*	1.00 (0.000)*	0.04 (0.769)
HDL-c	-0.24 (0.070)	-0.08 (0.546)	0.36 (0.004)*		0.36 (0.004)*	-0.45 (0.0003)*
VLDL-c	-0.30 (0.019)*	-0.23 (0.077)	1.00 (0.000)*	0.36 (0.004)*		0.04 (0.769)
LDL-c	0.17 (0.185)	0.83 (0.000)*	0.04 (0.769)	-0.45 (0.0003)*	0.04 (0.769)	

*Statistically significant ($p<0.05$). The respective p values are in the brackets.

The FBG concentration of the diabetic subjects was positively but significantly ($p<0.05$) correlated with nHDL-c (Non-high density lipoprotein cholesterol) of the subjects(Table 4.5). Similarly, the CRI-1(Castelli's risk index I), CRI-11(Castelli's risk index II), and AC (Atherogenic coefficient) of the subjects showed significant positive relationships with nHDL-c concentration.

Table 4.5: Correlation coefficient (p value) matrix of blood glucose concentration and atherogenic indices of diabetic subjects (n = 60).

	FBG (mg/dl)	nHDL-c (mmol/L)	CRI-I	CRI-II	AC	AIP
FBG		0.28 (0.027)*	0.25 (0.054)	0.22 (0.091)	0.25 (0.054)	-0.12 (0.365)
nHDL-c	0.28 (0.027)*		0.67 (0.000)*	0.61 (0.000)*	0.67 (0.000)*	0.21 (0.110)
CRI-I	0.25 (0.054)	0.67 (0.000)*		0.94 (0.000)*	1.00 (0.000)*	0.46 (0.0002)*
CRI-II	0.22 (0.091)	0.61 (0.000)*	0.94 (0.000)*		0.94 (0.000)*	0.48 (0.0001)*
AC	0.25 (0.054)	0.67 (0.000)*	1.00 (0.000)*	0.94 (0.000)*		0.46 (0.0002)*
AIP	-0.12 (0.365)	0.21 (0.110)	0.46 (0.0002)*	0.48 (0.0001)*	0.46 (0.0002)*	

*Statistically significant ($p<0.05$). The respective p values are in the brackets.

FBG (Fasting blood glucose) was found to be positively correlated with weight (Wt) (Table 4.6). BMI (Body mass index) correlated positively with Wt (Weight), WC (Waist circumference), HC (Hip circumference), WHtR (Waist to height ratio), SBP (Systolic blood pressure) and DBP (Diastolic blood pressure) but negatively correlated with Ht (Height) and WHR (Waist to hip ratio).

Table 4.6: Correlation coefficient (p value) matrix of blood glucose concentration and anthropometric indices of diabetic subjects (n = 60).

	FBG(mg/dl)	Ht (m)	Wt (kg)	WC (m)	HC (m)	AC (m)	BMI (kg/m^2)	WHR	WHtR	SBP (mmHg)	DBP (mmHg)
FBG		-0.25 (0.055)	0.38 (0.003)*	0.00 (0.997)	-0.15 (0.247)	0.16 (0.237)	0.18 (0.176)	0.16 (0.219)	0.16 (0.217)	-0.20 (0.132)	0.02 (0.878)
Ht	-0.25 (0.055)		0.28 (0.033)*	-0.17 (0.184)	-0.21 (0.155)	-0.02 (0.852)	-0.38 (0.003)*	0.04 (0.748)	-0.69 (0.000)*	-0.20 (0.118)	-0.22 (0.085)
Wt	-0.38 (0.003)*	0.28 (0.033)*		0.57 (0.000)*	0.73 (0.000)*	0.01 (0.921)	0.78 (0.000)*	-0.32 (0.013)*	0.27 (0.039)*	0.32 (0.012)*	0.15 (0.249)
WC	0.00 (0.997)	-0.17 (0.184)	0.57 (0.000)*		0.59 (0.000)*	0.21 (0.109)	0.64 (0.000)*	0.29 (0.025)*	0.83 (0.000)*	0.27 (0.036)*	0.22 (0.088)
HC	-0.15 (0.247)	-0.21 (0.115)	0.73 (0.000)*	0.59 (0.000)*		0.08 (0.538)	0.84 (0.000)*	-0.60 (0.000)*	0.55 (0.000)*	0.30 (0.020)*	0.17 (0.193)
AC	0.16 (0.237)	-0.02 (0.852)	0.01 (0.921)	0.21 (0.109)	0.08 (0.538)		0.08 (0.564)	0.12 (0.377)	0.18 (0.165)	-0.08 (0.528)	-0.15 (0.245)
BMI	0.18 (0.176)	-0.38 (0.003)*	0.78 (0.000)*	0.64 (0.000)*	0.84 (0.000)*	0.08 (0.564)		-0.36 (0.005)*	0.69 (0.000)*	0.45 (0.000)*	0.31 (0.017)*
WHR	0.16 (0.219)	0.04 (0.748)	-0.32 (0.013)*	0.29 (0.025)*	-0.60 (0.000)*	0.12 (0.377)	-0.36 (0.005)*		0.18 (0.168)	-0.07 (0.611)	0.01 (0.957)
WHtR	0.16 (0.217)	-0.69 (0.000)*	0.27 (0.039)	0.83 (0.000)*	0.55 (0.000)*	0.18 (0.165)	0.69 (0.000)*	0.18 (0.168)		0.31 (0.015)*	0.29 (0.027)*
SBP	-0.20 (0.132)	-0.20 (0.118)	0.32 (0.012)*	0.27 (0.036)*	0.30 (0.020)*	-0.08 (0.528)	0.45 (0.000)*	-0.07 (0.611)	0.31 (0.015)*		0.42 (0.001)*
DBP	0.02 (0.878)	-0.22 (0.085)	0.15 (0.249)	0.22 (0.088)	0.17 (0.193)	-0.15 (0.245)	0.31 (0.017)*	0.01 (0.957)	0.29 (0.027)*	0.42 (0.001)*	

*Statistically significant ($p<0.05$). The respective p values are in the brackets.

Serum GPx (Glutathione peroxidase) activity of the diabetic subjects was found to be negatively correlated with their TG (Triacylglycerol) and VLDL-c (Very low density lipoprotein cholesterol) concentrations.

Table 4.7: Correlation coefficient (p value) matrix of serum oxidative stress and lipid profile parameters of diabetic subjects (n = 60).

	GSH(µmol/L).	GPx (U/ml)	MDA (nmol/ml)	SOD (U/ml)	CAT (kU/l)
TC (mmol/L)	0.14 (0.279)	0.10 (0.456)	-0.25 (0.053)	-0.14 (0.275)	-0.04 (0.761)
TG (mmol/L)	0.06 (0.673)	-0.49 (0.0001)*	0.15 (0.257)	-0.08 (0.547)	0.16 (0.208)
HDL-c(mmol/L)	0.05 (0.719)	0.03 (0.825)	0.02 (0.876)	-0.10 (0.426)	0.09 (0.513)
VLDL-c(mmol/L)	0.06 (0.666)	-0.49 (0.0001)*	0.15 (0.257)	-0.08 (0.554)	0.16 (0.216)
LDL-c(mmol/L)	0.13 (0.339)	-0.14 (0.278)	-0.17 (0.197)	-0.10 (0.425)	-0.01 (0.934)

*Statistically significant ($p<0.05$). The respective p values are in the brackets.

There were no observed significant relationship between the oxidative stress and atherogenic indices of the diabetic subjects studied(Table 4.8).

Table 4.8: Correlation coefficient (p value) matrix of serum oxidative stress parameters and atherogenic indices of diabetic subjects (n = 60).

	GSH(µmol/L).	GPx (U/ml)	MDA (nmol/ml)	SOD (U/ml)	CAT (kU/l)
nHDL-c (mmol/L)	0.09 (0.478)	0.07 (0.615)	-0.22 (0.095)	-0.07 (0.619)	-0.08 (0.561)
CRI-I	-0.06 (0.624)	-0.02 (0.887)	-0.09 (0.483)	0.01 (0.917)	-0.09 (0.486)
CRI-II	-0.11 (0.408)	0.01 (0.964)	-0.01 (0.946)	0.06 (0.654)	-0.03 (0.794)
AC	-0.06 (0.624)	-0.02 (0.887)	-0.09 (0.483)	0.01 (0.917)	-0.09 (0.486)
AIP	0.08 (0.533)	-0.12 (0.346)	-0.09 (0.506)	-0.01 (0.936)	-0.03 (0.817)

*Statistically significant ($p<0.05$). The respective p values are in the brackets.

Significant ($p<0.05$) negative correlations were observed between arm circumference (AC) and GSH (Glutathione) concentration and CAT (Catalase) activity(Table 4.9).

Table 4.9: Correlation coefficient (p value) matrix of serum oxidative stress parameters and anthropometric indices of diabetic subjects (n = 60).

	WC (m)	HC (m)	AC (m)	BMI (kg/m^2)	WHR	WHtR	SBP (mmHg)	DBP (mmHg)
GSH	-0.08	-0.01	-0.28	-0.02	-0.06	-0.04	0.05	0.13
(µmol/L).	(0.563)	(0.938)	(0.032)*	(0.888)	(0.646)	(0.743)	(0.678)	(0.337)
GPx	0.18	0.12	0.04	0.13	0.06	0.16	-0.04	0.13
(U/ml)	(0.169)	(0.376)	(0.788)	(0.332)	(0.626)	(0.217)	(0.742)	(0.339)
MDA	0.04	-0.13	0.22	-0.19	0.22	0.11	-0.14	-0.04
(nmol/ml)	(0.776)	(0.312)	(0.092)	(0.153)	(0.094)	(0.411)	(0.273)	(0.766)
SOD	0.04	-0.17	0.17	-0.15	0.23	-0.02	0.03	0.14
(U/ml)	(0.784)	(0.202)	(0.199)	(0.263)	(0.082)	(0.865)	(0.8364)	(0.273)
CAT	-0.09	0.05	-0.28	0.03	-0.12	0.05	-0.06	0.04
(kU/l)	(0.477)	(0.695)	(0.033)*	(0.802)	(0.353)	(0.717)	(0.640)	(0.756)

*Statistically significant ($p<0.05$). The respective p values are in the brackets.

Significant negative correlations (Table 4.10) existed between TG (Triacylglycerol), HDL-c (High density lipoprotein cholesterol) and VLDL-c (Very low density lipoprotein cholesterol) concentrations and atherogenic indices, while LDL-c (Low density lipoprotein cholesterol) concentration was positively correlated with atherogenic indices (except AIP (Atherogenic index of plasma)). TC (Total cholesterol) was found to be positively correlated with only nHDL-c (non- HDL cholesterol)

Table 4.10: Correlation coefficient (p value) matrix of serum lipid profile and atherogenic indices of diabetic subjects (n = 60).

	TC (mmol/L)	TG (mmol/L)	HDL-c (mmol/L)	VLDL-c (mmol/L)	LDL-c (mmol/L)
nHDL-c	0.87	-0.37	-0.57	-0.37	0.91
(mmol/L)	(0.000)*	(0.003)*	(0.000)*	(0.003)*	(0.000)*
	0.25	-0.33	-0.92	-0.33	0.58
CRI-I	(0.052)	(0.010)*	(0.000)*	(0.010)*	(0.000)*
	0.18	-0.31	-0.91	-0.31	0.52
CRI-II	(0.165)	(0.016)*	(0.000)*	(0.016)*	(0.000)*
	0.25	-0.33	-0.92	-0.33	0.58
AC	(0.052)	(0.010)*	(0.000)*	(0.010)*	(0.000)*
	-0.04	-0.07	-0.49	-0.07	0.20
AIP	(0.736)	(0.619)	(0.0001)*	(0.613)	(0.135)

*Statistically significant ($p<0.05$). The respective p values are in the brackets.

There were significant ($p<0.05$) negative correlations (Table 4.11) between HDL-c (High density lipoprotein cholesterol) concentration and Ht (Height), HC (Hip circumference), BMI (Body

mass index), WHtR (Waist to height ratio), SBP (Systolic blood pressure) and DBP (Diastolic blood pressure) , while positive correlations were observed between the studied anthropometric indices and LDL-c (Low density lipoprotein cholesterol). Positive correlations were observed between TC (Total cholesterol) and Wt (Weight), WC (Waist circumference) and HC.

Table 4.11: Correlation coefficient (p value) matrix of serum lipid profile and anthropometric indices of diabetic subjects (n = 60).

	Ht	Wt	WC	HC	AC	BMI	WHR	WHtR	SBP	DBP
	-0.23	0.37	0.32	0.39	-0.07	0.23	0.10	-0.11	-0.21	-0.01
TC	(0.079)	(0.004)*	(0.012)*	(0.002)*	(0.598)	(0.084)	(0.470)	(0.417)	(0.105)	(0.970)
	-0.05	-0.07	-0.10	0.00	0.20	0.01	-0.06	-0.04	0.22	-0.04
TG	(0.691)	(0.581)	(0.453)	(0.975)	(0.127)	(0.969)	(0.627)	(0.775)	(0.089)	(0.748)
	-0.41	0.12	0.20	-0.33	0.18	-0.38	-0.16	-0.37	-0.33	-0.32
HDL-c	(0.001)*	(0.345)	(0.125)	(0.010)*	(0.167)	(0.003)*	(0.221)	(0.004)*	(0.009)*	(0.013)*
	-0.05	-0.07	-0.10	0.00	0.20	0.01	-0.07	-0.04	0.22	-0.04
VLDL-c	(0.689)	(0.578)	(0.448)	(0.976)	(0.124)	(0.969)	(0.620)	(0.772)	(0.088)	(0.748)
	-0.01	0.43	0.44	0.52	-0.07	0.41	0.14	0.31	0.27	0.20
LDL-c	(0.961)	(0.001)*	(0.000)*	(0.000)*	(0.590)	(0.001)*	(0.277)	(0.016)*	(0.037)*	(0.134)

*Statistically significant ($p<0.05$). The respective p values are in the brackets.

Significant positive relationships exist between HC (Hipcircumference), BMI, (Body mass index), WHtR (Waist to height ratio), SBP (Systolic blood pressure) and DBP (Diastolic blood pressure), on one hand and nHDL-c (Non-high density lipoprotein cholesterol) concentrations. CRI-I (Castelli's risk index1), CRI-II (Castelli's risk index II) andAC (Atherogenic coefficient) on the other. Similarly AIP (Atherogenic index of plasma) was positively correlated with BMI and WHtR (Waist to height ratio) only. The heights of the subjects were negatively correlated with their CRI-I, CRI-II, AC and AIP values.

Table 4.12: Correlation coefficient (p value) matrix of serum atherogenic and anthropometric indices of diabetic subjects (n = 60).

	Ht	Wt	WC	HC	AC	BMI	WHR	WHtR	SBP	DBP
	-0.02	0.37	0.37	0.48	0.15	0.38	0.16	0.27	0.34	0.16
nHDL-c	(0.907)	(0.004)*	(0.004)*	(0.000)*	(0.259)	(0.003)*	(0.225)	(0.035)*	(0.008)*	(0.209)
	-0.40	0.19	0.26	0.40	0.08	0.44	-0.19	0.40	0.38	0.39
CRI-I	(0.001)*	(0.153)	(0.049)*	(0.001)*	(0.536)	(0.000)*	(0.153)	(0.001)*	(0.003)*	(0.002)*
	-0.37	0.19	0.20	0.39	0.06	0.43	-0.24	0.34	0.38	0.42
CRI-II	(0.004)*	(0.140)	(0.133)	(0.002)*	(0.637)	(0.001)*	(0.062)	(0.007)*	(0.003)*	(0.001)*
	-0.40	0.19	0.26	0.40	0.08	0.44	-0.19	0.40	0.38	0.39
AC	(0.001)*	(0.153)	(0.049)*	(0.001)*	(0.536)	(0.000)*	(0.153)	(0.001)*	(0.003)*	(0.002)*
	-0.47	-0.06	0.10	0.23	0.11	0.26	-0.14	0.34	0.10	0.13
AIP	(0.000)*	(0.645)	(0.451)	(0.077)	(0.389)	(0.049)*	(0.301)	(0.008)*	(0.468)	(0.340)

*Statistically significant ($p<0.05$). The respective p values are in the brackets.

4.1.9 Dyslipidaemia distribution

Table 4.13 shows that majority of the diabetic subjects had abnormally high prevalence of dyslipidaemia with all the subjects (100%) having elevated LDL-c, and only 35% having abnormally low HDL-c concentration.

Table 4.13 Dyslipidaemia distribution in the diabetic subjects (n = 60).

Dyslipidaemia	**Number (%) of patients**	**Cut off Point (Reference)**
Hypercholesterolemia	47(78.3)	TC > 5.2mmol/L (NCEP, 2002)
Hypertriglyceridemia	55(91.7)	TG > 1.7mmol/L (NCEP, 2002)
Low HDL-c	21(35.0)	HDL-c < 1.04mmol/L(NCEP, 2002)
Increased VLDL-c	55(91.7)	VLDL-c > 0.77mmol/L (MedlinePlus, 2014).
Elevated LDL-c	60(100)	LDL-c >3.34mmol/L(NCEP, 2002)

4.1.10 Abnormal atherogenic indices distribution

Over 60% of the diabetic subjects had abnormally high levels of atherogenic predictor indices in the range of subjects with abnormal CRI-II > AIP >nHDL-c > AC, with prevalence of abnormality in CRI-I being least.

Table 4.14. Diabetic subjects with high (abnormal) values of atherogenic indices

Atherogenic indices	Number (%) of patients	Cut off value (reference)
nHDL-c	43(71.6)	>4.0mmol/L (HEART UK, 2016)
CRI I	16 (26.7)	>6.0 (HEART UK, 2016)
CRI II	54(90.0)	>3.5 (Shimomura and Wakabayashi, 2013).
AC	39 (65.0)	>3.49 (Al-Qaicy, 2015).
AIP	49(81.7)	>0.214 (Dobiášová*et al.*, 2011; Al-Qaicy, 2015)

4.2 Discussion

Type 2 diabetes, a metabolic disorder characterized by hyperglycaemia, is usually associated with obesity, insulin resistance, dyslipidaemia (cardiovascular risk), endothelial dysfunction, diabetic complications, as well as the presence of increased oxidative stress (Manjulata, Tarun, Ishwar, Neelima, Veena, Satish and Vijay,2013; Tangvarasittichai, 2015). Hence routine monitoring of blood glucose, serumlipid profile and the level of oxidative stress in type 2 diabetics is recommended to assess the progress of the disease, the risk of cardiovascular diseases and diabetic complications which will aid in effective management of the disease (Igwe, Ibegbulem, Ukwamedua & Ikaraoha, 2008).

4.2.1Baseline Information of the Diabetic Subjects

Equal number of female 30(50%) and male 30(50%) subjects with type 2 diabetes mellitus (T2DM) were enrolled in this study, with most of the patients (81.7%) residing in urban area. The location of the hospital under study, Awka, the capital of Anambra State was responsible for higher number of urban dwellers among the patients. In addition, western life style (a type 2 diabetes risk factor) is inevitable in a fast growing city like Awka, which might have contributed to the incidence of type 2 diabetes in the area. The hospital location also reflected in educational status of the diabetic subjects as only 23.3% had no formal education. Meanwhile, out of the 76.7% that had formal education, 31.7% obtained educational qualifications higher than secondary education. This study showed that majority of the patients residing in the urban area were males(46.7%), and this, as well as being more educated (49.0%) than the females (26.7%),

could explain why most of the civil servants(23.3% out of 33.3%) and traders (8.3% out of 11.7%) were males, while all the farmers (10.0%) were females.

The results of this study showed a steady rise in the prevalence ofT2DM with age. Worsening of insulin resistance with age, increased physical inactivity, and improved longevity of T2DM patients due to better healthcare could be the possible reasons (Burattini, Di Nardo, Boemi & Fumile2006).We observed equal prevalence rates of T2DM (26.7% males and 26.7% females) among the gender groups of 60-70 years old. Our study also showed equal incidence without age factor. Equal incidence of T2DM among various adult age groups had been reported earlier (Wild *et al,* 2004).However, a previous study in North Kerala, India,reported male DM predominance (Valliyot, Sreedharan, Valliyot & Jayakumary-Muttappallymyalil, 2014), which is in agreement with the present study for the ages of 50-59.In this present study, 10% females and 6.7% males were between the ages of 40-49 years of age, suggesting that females avail themselves early enough for diagnosis than men. In the traditional African family setting, men unlike women tend to resist or argue about going for medical checkup until it becomes inexpedient, under persuasion of their family members (Igwe *et al.*,2008).

More numbers of the subjects (55%) were employed and this is expected in an urban city. The observed relatively high percentage of unemployed (45%) among the subjects may be attributable to ill health and aging (53.4% of the subjects were within 60-70 years old) and may explain the characteristic of T2DM being disease of the adult (Igwe, Duru, Ukwamedua & Ikaraoha, 2007). Also, 51.7% of the patients were on diabetic diet, which is close to the percentage of employed patients (55%). It will not be out of place to assume that employment availed them the financial support to be able to afford diabetic meals, which are usually more expensive than the usual diet of carbohydrate-based staple food in this region. Furthermore, the educational status of most patients must have helped them in understanding the importance of feeding on diabetic meals. In this study it was observed thatvegetable consumption by most diabetic subjects is based on the usual practice of cooking most meals with vegetables by people of this region. Only 16.7% of the T2DM patients, out of which 10% were females, realized the need to increase the intake of vegetables to improve their health condition by making it a daily practice. This is probably due to the fact that most females in this part of the world eat home prepared meals, unlike males that often eat outside their homes, and therefore may be prone to eating more of junk foods, rather than vegetables containing meals.

About 56.6% of the diabetics (33.3% females and 23.3% males) in this study have had T2DM for 2-10 years duration (Table 4.2). This is based on the information on years of diagnosis/treatment obtained from the patients. However, the possibility of late diagnosis cannot be ruled out completely. Out of 15% of the patients that had over 10 years diabetes duration, 10% were males while 5% were females, implying that the males may possess tendency to live longer with diabetes than females. It has been earlier reported that total life expectancy increase among diabetics is stronger in men than women (Muschik, Tetzlaff, Lange, Epping, Eberhard & Geyer, 2017).

In this study, 28.3%of the diabetic subjects also had hypertension.Hypertension and diabetes are both end results of the metabolic syndrome and may, therefore, develop one after the other in the same individual (Bernard&Chao, 2012). Male patients (18.3%) showed more tendency to co-morbidity with hypertension than females (10%).This demands for more research, since a higher prevalence of hypertension in T2DM women had been previously reported (Collier, Ghosh, Hair, &Waugh,2015). However, the variance could be adduced to differences in lifestyle as well as efficacy and gender-associated side effects of anti-DM drugs.All the subjects were on diabetic drugs, with 11.7% of the subjects being on additional drugs namely antibioticsand hypertensive drugs. Drugs may affect the results of biochemical parameters, for examples, the use of α-glucosidase inhibitors are effective at reducing fasting plasma glucose (FPG) levels (Michael, 2016) and the activity oflipoprotein lipase (LPL) could be increased by drug therapy(Goldberg, 2001).

After diagnosis, some of the patients developed additional symptoms over time:feet numbness/leg pain(43.3%),chest pain (18.4%) and increased weakness (21.7%), which could be early signs of diabetic complications, probably diabetic neuropathy (feet numbness), diabetic nephropathy(weakness and leg pain) and heart attack (chest pain) (Marks, 2017).There was a marked significant difference between male (51.7%) and female (31.7%) patients that developed additional symptoms. Arnetz, Ekberg, &Alvarsson*(*2014) reported that men with T2DM appear to suffer more microvascular complications. Majority of the male patients that participated in the present study were civil servants, and as such were predisposed to sedentary lifestyle due to long sitting time in offices, which may also contribute to higher percentage of complications.

In the present study, 73.3% of the subjects had family history of diabetes, which further supported the evidence of a strong inheritable genetic connection in type 2DM (Rother,

2007).Our results showed unequal prevalence in family history of T2DM (43.3% males and 30.0 % females), whereas equal prevalence of family history was observed by Valliyot *et al* (2014).Conversely, higher prevalence in family history of female T2DM patients was reported by Annis, Caulder, Cook & Duquette (2005).Thus more research with respect to role of paternal/maternal linage, sibling, and age on T2DM may give more insight into the observed gender-based differences in family history inT2DM.The frequency of regular blood glucose monitoring varied in diabetic subjects under study, with the most frequent (≤ 3 times a week) done by only 20% of the subjects, while 70% did their routine check once in 2 weeks. Usually, the number of tests per day or per week will differ for everyone with diabetes, and should be worked out in consultation with a healthcare provider.People recently diagnosed or starting a new diabetes drug may need test of up to three or four times every day, while those with well-controlled blood glucose may only need 2 or 3 days every week(NPS, 2016).Glycaemic control is essential to diabetes management.Patient's self-monitoring of blood glucose (SMBG) is one of the primary techniques recommended by American Diabetes Association (ADA) for health providers and patients to assess the effectiveness of the management plan on glycaemic control.This may be helpful to guide treatment decisions and/or patient self-management for patients using non-insulin therapies(like the subjects under study).Thefrequency of SMBG and timing should be dictated by the patient's specific needs and goals(ADA, 2014).

Patients with systolic blood pressure of ≥130 mmHg were 41.7% and diastolic blood pressure of ≥90 mmHg were 13.3%, some of which were among the 28.3% hypertensive diabetics, while some were at risk of developing hypertension (ADA, 2016). Approximately 63.3% of the subjects had BMI of ≥ 25kg/m^2, out of which 40% were obesed. This result suggests future diabetic complications including cardiovascular disease, CVD (Huxley *et al.,* 2010), in these diabetic subjects. Changes in dietary pattern and regular exercise may be of immense help for weight control in these patients to avoid future complications.

4.2.2 Changes in fasting blood glucose (FBG) concentration of the diabetic subjects

Significant increase was observedin FBG concentration of the diabetic subjects compared to the control both within and between male and female groups.In diabetes mellitus, excess sugar (glucose) is found in blood (Mandal, 2012).Rise in blood glucose concentration (hyperglycaemia) beyond its renal threshold (about 10 mmol/L) could lead to incomplete

reabsorption of glucose in the proximal renal tubule causing glycosuria, polyuria and polydipsia which may progress to orthostatic hypotension and dehydration(Preeti, 2014). Hyperglycaemic state triggers metabolic alterations (enhanced polyol activity, causing sorbitol and fructose accumulation; increased formation of advanced glycation end products; activation of protein kinase C and nuclear factor κB; and increased hexosamine pathway flux) which occur mainly in endothelial cells and can result in endothelial dysfunction and contribute to vascular damage (Giugliano, Ceriello & Esposito2008). Also prolonged hyperglycaemia in diabetes can result in diabetic ketoacidosis (DKA), due to excess fatty acid oxidation to compensate for energy needs as a result of decreased glucose utilization (Chhabra, 2015).

4.2.3Changes in lipid profile levels of the diabetic subjects

The lipid parameters, TC, TG, LDL-c and VLDL-c were significantly increasedin the diabetic subjects as compared to the control subjects, while HDL-c concentration was significantly decreased in diabetics compared to the controls.Similar trend of observations were seen among the male and female diabetic subjects. These findings are in agreement with similar earlier studies conducted in different parts of the world (Kumar *et al*, 2014; Narasimhaswamy,Ravi & Neema, 2014).Kumar *et al* (2014) reported that common lipid abnormalities during diabetes-induced dyslipidaemia are hypercholesterolaemia, hypertriglyceridaemia and elevated LDL-c, which are in agreement with our findings; however they also observed slight insignificant increase and decrease in VLDL-c and HDL-c, respectively. Narasimhaswamy *et al*(2014) showed that cholesterol, triglyceride, VLDL, LDL were significantly higher, while HDL levels were significantly lower in type 2 diabetes mellitus cases as compared to controls.

Increased levels of serum cholesterol as compared to controls (Figure 4.2) could be due to the following reasons as reviewed byNarasimhaswamy *et al* (2014).

i. Impaired, over stimulation of HMG - CoA reductase enzyme by glucagon which is rate limiting for cholesterol synthesis.

ii. Defective catabolism of cholesterol into bile acids also leads to more cholesterol in blood.

iii. More VLDL in plasma causes hypercholesterolaemia because VLDL carries about 20% of its total lipid content as cholesterol.

iv. Cholesterol absorption in type 2 diabetes mellitus subjects is significantly lower while cholesterol synthesis is significantly higher.

Lower HDL-c concentration in diabetics (Figure 4.3) could be multifactorial.Kinetic studies using radioisotopes and stable isotopes have demonstrated that the decrease in HDL-cholesterol in patients with type 2 diabetes is due to increased catabolism of HDL-c. The activity of hepatic lipase, the enzyme controlling HDL catabolism, is augmented in insulin-resistant states, which is likely to be responsible for the observed increase in HDL catabolism. It is suggested that the increased pool of triacylglycerol-rich lipoproteins (mainly VLDL), observed in type 2 diabetes, promotes cholesterol ester transfer protein (CETP)-mediated triacylglycerol enrichment of HDL particles and, as a consequence, enhances HDL catabolism, since triglyceride-rich HDLparticles are very good substrates for hepatic lipase (Vergès, 2015).The surface lipid from triglyceride-rich particles that are transferred to HDL during VLDL and chylomicron lipolysis increases HDL lipid content.Defective lipolysis, due to decreased lipoprotein lipase activity in insulin resistant state, leads to reduction in HDL production through this process(Goldberg, 2001).The present study showed that diabetic females had significantly higher HDL-c than the male diabetics (Figure 4.3). Some other studies also reportedhigher HDL-c in female than male diabetics, though not statistically significant. Mahato, Gyawali, Raut, Regmi, Singh, Pandeya &Gyawali*(*2011) reproted 46.52 ±0.97 and 45.93±0.61mg/dl as the mean values of HDL-c in female and male subjects respectively. Another work found the mean values of HDL-c in females/males to be 44.9 ± 1.3 /40.8 ± 1.2mg/dl (Ozder, 2014), while Kumar *et al*,(2014) reported 31.5 ± 6.7/ 30.2 ± 7.4mg/dl of HDL-c in female/male diabetics.In addition, the CVD risk cut off values for HDL-cgiven by the US National Cholesterol Education Program (NCEP) guidelines shows that HDL-c concentrationis usuallyhigher in women than men (NCEP, 2002).

Increased serum triacylglycerol levels in the diabetic subjects studied (Figure 4.4) may be largely due to release of non-esterified fatty acids (NEFA) from adipocytes which is transported to the liver.This may be due to reduced suppression of lipolysis caused by insulin resistance. Moreover, type 2 diabetes is associated with visceral adiposity, which increases the delivery of NEFA directly to the liver via portal circulation, therebystimulating synthesis of triglycerides in hepatocytes. The increased hepatic triglycerides are packaged into lipoprotein particles (chylomicrons and VLDL-triglyceride-rich lipoproteins (TRL). Thus,hypertriglyceridaemia potentially results from increased production of VLDL, reduced clearance of TRL or a combination of both mechanisms. The reduced clearance of TRL is as a result of reduced lipoprotein lipase (LpL) activity(hydrolyzes triglyceride rich lipoproteins), which is regulated by

insulin(Alan, 2012; Pang*et al,* 2014).Thus, impairment of LpL activity in type 2 diabetes due to insulin resistance, amplifieshypertriglyceridaemia.

The observed increase in VLDL-c concentration in our study may be as a result of increased fatty acid transport to the liver. This is because of increased lipolysis in type 2 DM (Sondergaard *et al.,* 2012).Both increased production and delayed catabolism of VLDL are responsible for the increased VLDL pool (Goldberg, 2001).Not all VLDL are equally likely to be converted to LDL. A greater proportion of large lighter VLDL(due to greater triglyceride content) returns to the liver without complete conversion to LDL(Goldberg, 2001;Vergès, 2015).Furthermore, the usual suppressant effect of insulin on postprandial VLDL (more specifically, $VLDL_1$) production is blunted by hepatic insulin resistance (Sondergaard*et al.,* 2012).

Higher serum LDL-c concentration observed in the diabetics(Figure 4.6) may be due to raised VLDL, because VLDL is the precursor of LDL catalysed by lipoprotein lipase (LPL).The activity of LPL could be increased by weight loss, fibricacid drug therapy, and treatment of diabetes (Goldberg, 2001). Also, as stated byNarasimhaswamy *et al.* (2014), LDL receptor is up regulated by insulin, but in type 2 diabetes mellitus due to insulin resistance, there is less uptake of LDL.

4.2.4 Atherogenic indicesof the diabetic subjects

Strong significant elevations were observed in all the atherogenic indices (non - HDL cholesterol, CRI 1, CRI 11, AC and AIP) studied in diabetic subjects when compared to controls. This finding further supports severalreports of theusefulness of these indices for identifying high risk individuals for CVD and effective drug management, thus complementing the existing lipid profile tests (Bhardwaj *et al,* 2013; Patil,Jayaram, Meera & Kantharaj2015; Meenakshi, Mauchumi & Anindita, 2015; Nimmanapalli*et al,* 2016).

According to the National Cholesterol Education Program (NCEP ATP III), the level of non-HDL-cin the management of CVD should be targeted at < 3.35mmol/L(NCEP, 2002). The present study showed that the mean value of non-HDL-c in the study population was 4.48 mmol/L (Figure 4.7), which is higher than the NCEP ATP III recommended value, suggesting an increased risk of developing CVD in these patients. Also in the present study, there was a significant increase in the n-HDL-c of the patients when compared to the mean value of the non-diabetics (1.95mmol/L).Nimmanapalli*et al.*(2016)found a significant increase in the levels of

non-HDL-cholesterol in both T2DM patients with (186.76±16.41mg/dl) and without (137.84±18.41mg/dl) complications when compared to controls(121.8±21.02 mg/dl).Sreevani*et al.* (2016) also reported anincreased level of non HDL-c in T2DMpatients (166.6±32.1mg/dl) compared to that of controls(98.8±22.6mg/dl). Non HDL-c is recommended as the second target of therapy after LDL-c especially in individuals with hyper-triglyceridaemia (NCEP, 2002). The observation of 91.7% of patients having hyper-triglyceridaemia in the present study collaborates the recommendation of NCEP (2002). Non HDL-c concentration wss higher in male than female diabetics in this study probably because of higher HDL-c level in female diabetics with no significant difference observed in the TC concentrations of both groups.

A study by Bhardwaj *et al.*(2013) reported that CRI-I calculated as the ratio of TC to HDL-c, and CRI-II as LDL-c/HDL-c, were found to be significantly higher in diabetics (CRI-I= 5.48±0.23; CRI-II= 3.18±0.16) compared to those of controls (CRI-I= 3.80±0.16 ; CRI-II= 2.37±0.14).This is in agreement with the results of the present study in which diabetic subjects had significantly higher CRI-I (5.31 ± 1.19) and CRI-II (5.29 ± 2.07) values compared to controls (CRI-I = 2.27 ± 0.70 ; 1.55 ± 0.63)(Figures 4.8 and 4.9 respectively). Similarly, Patil*et al* (2015)reported significant increases in CRI-1 (4.79 ± 1.20) and CRI-11(2.74 ± 0.88) in diabetics, when compared to non-diabetics(3.80 ± 0.68 and 2.26 ± 0.59).However, Patil*et al*(2015)did not observe a significant difference in TC and LDL-c levels between the patients and controls, contrary to the results of the present study. They noted that the ratios(CRI-I and CRI-II) based on these parameters showed a significant difference between the two groups, hence they suggested the relevance of these ratios over individual lipid parameters. However, Sreevani, Rajyalakshmi, Aruna, Gandham & Vodelu, (2016) found statistically significant increases in TC, LDL-c, CRI-Iand CRI-IIof type 2 diabetes mellitus patients compared to controls as was also observed in our study.Our results showed that these indices were also significantly higher in males than female diabetics which could be due to higher HDL-c in female diabetics whereas no significant difference in TC and LDL was found between the groups. There is need for more studies on this to elucidate the basis for gender differences in CRI-Iand CRI-II.

Significantly higher atherogenic coefficient (non-HDL-c/HDL-c) was observed in diabetics than controls in this study. Similar study among Caucasians reported significant elevation in atherogenic coefficient(AC) in both T2DM patients with and without complications when compared to controls (Nimmanapalli*et al.*,2016). According toBhardwaj*et al.*(2013), AC

calculated as non-HDL-c /HDL-c or TC-HDL-c /HDL-c is a measure of cholesterol in LDL, VLDL, and IDL fractions in relation to good cholesterol or HDL-c. This implies that AC represents the atherogenic potential of the entire spectrum of lipoprotein fractions and hence an indicator of CVD risk. The level of AC was significantly higher in males than female diabetics in the present study. This is contrary to the report of Ahmed (2015) in which the mean values of AC in male and female diabetics were not significantly different. However, it is important to note that in the reported study (Ahmed, 2015), HDL-c level was higher in males than in females, contrary to the observations of the present research work.The gender variation in the level of AC in the present study could be attributed to differences in educational status, residence or occupation of the female diabetic subjects as compared to the males. Majority of the males were more educated, residing in towns and involved in civil service jobs with its associated sedentary life style than the females that were less educated, majorly rural farmers and petty traders with their attendant active and strenuous life style.

As in the current study, Bhardwaj*et al.*(2013) observed that atherogenic index of plasma (AIP) was significantly higher in diabetic subjects as compared to controls. Similarly,Patil*et al.*(2015) alsoobserved that AIP was significantly higher in diabetics when compared to controls. Results of the present study show that AIP mean value of the diabetics was positive and significantly higher than that of the non-diabetics.This suggests that the non-diabetics in the present study were not at risk of developing cardiovascular diseases. This deduction is based on the outcome of aprevious study which involved a total of 1433 individuals with various risks of atherosclerosis (including type 2 diabetes),in which it was reported that the values for AIP increased significantly with increasing atherogenic risk and with AIP ranging from −0.24 in normal controls to 0.51 in worst cases (Dobiášová&Frohlich, 2001).This means that individuals with AIP values of -0.24 are at the lowestatherogenic risk level, as is the case with non-diabetics in our study. The results also revealed that AIP of the male subjects was non-significantly greater than that for females. On the contrary, Ahmed (2015)reported higher AIP in female subjects than males, but with statistically no significant difference.

4.2.5 Changes in oxidative stress parameters of the diabetic subjects

There was a significant decrease in the reduced GSH concentration of the diabetic subjects as compared to non-diabetic subjects, which is in agreement with earlier studies (Sekhar*et*

al.,2011;Calabrese *et al.*,2012). Calabrese *et al.*(2012) reported a significant decrease in the content of reduced GSH in the type 2 diabetic samples compared with the control samples. According toSekhar*et al.*(2011),subjects with poorly controlled diabetes had 73.8% lower erythrocyte-reduced glutathione concentration than non-diabetic subjects.Similarly, Kalkan &Suher(2013) reported that GSH values was lowest in subjects with type 2 DM, higher in subjects withimpaired glucose tolerance (IGT), and highest in normal subjects. Results of the present study, in accordance with the above reportsindicated that patients with type 2 diabetes have lower concentrations of GSH, which increases the susceptibility of cells to the damaging effects of reactive oxygen species (ROS). Glutathione (GSH), a tripeptide, γ-L-glutamyl-L-cysteinylglycine, is the most abundant non-protein thiol that defends against oxidative stress by reducing hydrogen peroxide level in a state of oxidative stress(Lu, 2013).Hyperglycaemia increases glucose flux through the polyol pathway which decreases the intracellular levels of reducednicotinamide adenine dinucleotide phosphate (NADPH).This consequently impairs the regeneration of the reduced glutathione (GSH) since glutathione reductaserequires NADPH to regenerate GSH from GSSG. As GSH level reduces, cellular antioxidant capacity is compromised, resulting in elevated levels of ROSs that can attack macromolecules and induce oxidative damage (Liang-Jun, 2014).

Superoxide dismutase (SOD) activity measured in this study significantly decreased in diabetic subjectsthan non-diabetic subjects. Manjulata, Tarun, Ishwar, Neelima, Veena, Satish &Vijay (2013)reported a decrease in serum SOD activities of diabetics compared to controls.Another studyrevealed that SOD activity of type 2 diabetics was significantly lower than that of the control subjects, and it also decreased with increasing duration of type 2 DM (Briggs, Brown, Elechi-Amadi, Ezeiruaku & Nduka, 2013).The observed reduction could be linked to increased production of ROS(Briggs *et al*, 2013) and progressive glycation of enzymatic proteins. About 50% of SOD in erythrocytes of diabetic patients is glycated, resulting in low activity of SOD(Manjulata *et al*, 2013).

The concentration of MDA was significantly higher in diabetic subjects in comparison with the non-diabetics, suggesting an increase in lipid peroxidation and an augmented production of reactive oxygen and nitrogen species (RONS) in such patients (Savu, Ionescu-Tirgoviste, Atanasiu, Gaman, Papacocea & Stoian, 2012). Similar outcome, that is increased lipid peroxidation, was reported by Bandeira *et al.* (2012)in diabetic patients, which shows a close

relationship between high glycaemic levels and oxidative stress in diabetes mellitus.The result of present study also corroborated the mean MDA values previously reported in type 2 diabetics and controls respectively (Moussa, 2008). The observed increase in lipid peroxidation in the diabetic subjects is also an indication of decline in defence mechanisms of enzymatic and non-enzymatic antioxidants (Saddala *et al.*, 2013).

Glutathione peroxidase (GPx) activity significantly decreased in diabetic subjects than non-diabetic subjects. GSH is a substrate and cofactor of GPx, hence the low activity of GPx could be directly explained by the low content of GSH found in diabetics in this study. It could also be as a result of enzyme inactivation caused by severe oxidative stress or through glycation governed by prevailing high glucose concentration (Kewal, 2010; Gawlik *et al.*, 2016).

A significant decrease in catalase (CAT) activity was observed in the diabetics than non-diabetic subjects. This observation is in agreement with the report of Anees, Parveen, Mohammed, & Ishaq (2014), who noted that catalase activity in T2DM cases was reduced compared with non-diabetics. Catalase is an important antioxidant enzyme which protects the cells from toxic effects of hydrogen peroxide, generated during cell metabolism. Patel and coworkers had earlier reported that hyperglycaemia increases hydrogen peroxide production, hyperpolarizes mitochondrial membrane, and down regulates CAT gene expression(Patel *et al*, 2013).

4.2.6 Anthropometric indices of the diabetic subjects

It was observed that the BMI mean value ofthe diabetics was significantly higher than that of the non-diabetics. This observation is in consonance with the report of Lotfi *et al*(2014), who noted an increase in the BMI mean value of type 2 diabetics compared to controls. Our result also showed that the females had significantly higher BMI values than males for both diabetics and non-diabetics studied. Higher BMI in females than males was also reported in diabetics by Adunbiola (2014) and Devang *et al.* (2016).Similarly, Lotfi *et al*(2014) also observed higher BMI in femalesthan male diabetics.These results suggest a need for separate BMI cut off points for gender groups. Measuring BMI from a diabetes diagnosis perspective has a clinical significance of identifying individuals who may have undiagnosed diabetes or may be at increased future risk for diabetes (Hsu,*et al.*, 2015), or at increased risk of diabetes complications such as hypertension and heart disease (Diabetes Uk, 2017). A review of over 12,000 people in the United States, published in 2014, showed that people with a BMI of 25-29.9 kg/m²had a 50%

increased risk of diabetes compared to people with a BMI of 18.5-24.9kg/m²(Diabetes Uk, 2017). Also, measuring BMI is important for managing diabetes for the purpose of weight control (Hsu, Araneta, Kanaya, Chiang & Fujimoto, 2015). In other words, it helps in setting a weight loss goal which can be of immense benefit in lowering the risk of diabetes complications.

Our results showed that the diabetics had significantly higher WC, HC, WHR and WHtR than non-diabetics. These observations are consistent with the previous report that that both men and women with DM have a higher rate of central obesity (defined by increase in WC, WHR and WHtR) than non-diabetic subjects (Bhowmik,Munir, Ahmed, Siddiquee, Diep, Wright . . .& Hassan,2014).In the same vein, Dhakal,Singh, & Kumar(2015) reported that type 2 diabetics have significantly higher WHR ratio than controls. Lotfi *et al.*(2014) observed that among diabetics, the mean values of WC and WHR were significantly high compared to those of non-diabetics. According to Himabindu*et al.*(2013), the mean values of WC and WHtR in type 2 diabetics are usually above the threshold cut off values. The observed increases in these anthropometric indices indicate central obesity and abnormal distribution of fat in the body of these subjects. Type 2 diabetic subjects are most likely to have impaired WC and WHtR because abdominal obesity has been shown to be a significant risk factor for the development of type 2 diabetes.Stronger associations have been reported between anthropometric markers that reflect abdominal obesity (i.e. WC and WHtR) and incidence of T2DM than for BMI and weight (Hartwig*,* Kluttig, Tiller, Fricke, Müller, Schipf . . . & Greiser, 2016). From the results of our study, WC was significantly higher in female than male subjects, which is the same with the outcome of cross-sectional study of type 2 DM patients attending an outpatient clinicat Tamale, Ghana(Mogre,Abedandi&Salifu,2014). HC and WHtRwere also significantly higher in females than male diabetic subjects. These observations are in agreement with the findings of Adunbiola(2014), who reported higher HC and WHtR values in diabetic females.

It has been suggested that arm circumference (AC) can be used as an alternate index to measure obesity as it correlates very well with BMI (Devang*et al*, 2016).The AC of the diabetics in the present study was significantly higher than that of the non-diabetics.Devang*et al.* (2016)had earlier observed a non-significant increase in AC of diabetics compared to non-diabetics, whileFarah, Joyce, Kennelly, Daly & Turner (2010) observed a significant increase inAC in diabetic women compared to non-diabetic women.

4.2.7 Changes in blood pressures of the diabetic subjects

Diabetic subjects in the present study showed significantly higher levels of both systolic blood pressure (SBP) and diastolic blood pressure (DBP) when compared to non diabetic subjects. Devang*et al*, (2016) reported a significant increase in SBP of diabetics, but a non significant increase in DBP of diabetics compared to those of non-diabetics.Diabetes predisposes the arteries to atherosclerosis (narrowing of the arteries), which consequently causes high blood pressure (Bernard & Chao,2012). A recent study also suggested that sugar (glucose) is worse than salt in elevating blood pressure (Mercola, 2015).Our results showed that a slight significant increase was also noted in DBP of diabetic females than male diabetics. Similar findings werereported byDevang*et al.*(2016),who observed a significant increase in both SBP and DBP of the female diabetics compared to their male counterparts. Collier *et al.*(2015) also reported a higher prevalence of hypertension in type 2 diabetic women.

Possible explanations are: (1) That the medication used to treat high blood pressure is not as effective in women as in men; (2) That the pathophysiology of hypertension is different in men and women and thus the targets being treated are missed with existing/conventional therapy (Maric, 2011). It is however surprising that in non-diabetic women, blood pressure levels are lower compared with age-matched non-diabetic men, at least until menopause (Ong, 2007), which implies that diabetes eliminates the protective effect of the female sex with respect to hypertension (Maric, 2011).

4.2.8 Correlation studies of parameters of the diabetic subjects

Increase in fasting blood glucose (FBG) is a major hallmark of diabetes mellitus. The results of FGB significantly correlated positively with MDA but negatively with GSH **(**Table 4.3). These findings point to the fact that severity of oxidative stress parallels the degree of rise in blood glucose in patients with type-II diabetes mellitus(Bandeira *et al.*, 2012; Malam, Amin, Zala, Navadiya, Dhruv Patel & Patel,2016). MDA and GSH therefore appear to be reliably deranged markers in hyperglycaemia. MDA, a primary biomarker of free radical-mediated lipid damage and oxidative stress (Shodehinde & Oboh, 2013), was also positively related to SOD activity of the diabetic subjects (Table 4.3). SOD plays important protective roles against cellular and histological damages that are produced by ROS (Davari *et al.*, 2013).Thus; the positive correlation observed suggests that the activity of SOD increases in response to lipid peroxidation induced by ROS. On the other hand, it has been reported that peroxyl radicals can abstract

hydrogen from lipids, producing hydroperoxides that further propagate the free-radical pathway (Lobo, Patil, Phatak, & Chandra, 2010).The SOD anti-oxidative reaction produces H_2O_2 which could yield hydroxyl radicals through Fenton reaction (El-Bahr, 2013). The hydroxyl radical eventually leads to production of peroxyl radicals, and hence increases lipid peroxidation (elevated MDA).

The present study showed significant negative correlation of FBG with TG and VLDL-c, and no significant correlation with other lipid profile parameters. This observation partially disagrees with the report of Ozder (2014) in which FBG significantly and positively correlated with both TC and TG. LDL-c was positively related to TC but negatively correlated with HDL-c of the subjects in this study. Lam,Munro& Siu(1990) reported a highly significant correlation between serum total cholesterol (TC) and LDL-c in Chinese diabetic subjects. This indicates that serum total cholesterol measurement can be used as the initial screening to identify individuals who may need lipoprotein analysis. A negative relationship between LDL-c andHDL-c have also been observed in other studies (Ozder,2014; Joshi, Bhattacharya, Kar, Yadav, Sah& Pokhrel, 2014). Hence, the results obtained from the present study have confirmed the previous observations of increased LDL and decreased HDL cholesterol levels in diabetic patients (Joshi *et al.*, 2014).

The FBG concentrations of the diabetic subjects were found to be positively and significantly correlated with nHDL-c, which is in agreement with previous reports (Thambiah, Samsudin, George, Zahari Sham, Lee, Muhamad . . . & Mohamad, 2016). Since nHDL-c was significantly associated with FBG, whereas there was no association between LDL-c and FBG, this result supports previous hypothesis thatnHDL-c (TC - HDL-c) is a better predictor of CVD risk than a simple measure of LDL-c in type 2 diabetes mellitus (Warjukar, Ghangale & Iyer, 2016). Non-HDL-c was also found to have positive relationships with CRI-I, CRI-II and AC of the diabetic subjects. Hence nHDL-c, as well as theseindices are useful for screening and identifying high risk individuals for CVD (Adu *et al.*, 2015; Patil *et al.*, 2015).

The FBG of the diabetic subjects have positive significant correlation with their weight (Wt), but non-significantly related to BMI. Previous studies in Caucasian also reported a positive correlation between FBG and BMI (Onyesom,Oweh, Etumah & Ifie,2013). Many studies have indeed buttressed the importance of BMI (weight/ height^2) as an indicator of overweight and obesity in individuals who are at an increased risk of type 2 diabetes and its complications

including CVD (Huxley *et al*, 2010). Our results also showed thatBMI correlated positively with Wt, WC, HC, and WHtR, but negatively with Ht and WHR.WC, HC; and WHtRhave higher sensitivity and specificity for identifying diabetic individuals at increased risk of CAD (coronary artery disease)(Kaur,Sharma & Singh, 2014),thuscomplementing the use of BMI.The negative correlation observed between BMI and WHR may be due to the fact that WHR reflects abdominal adiposity, rather than overall adiposity as in the case of BMI, and have been suggested as being superior to BMI in predicting CVD risk (WHO, 2011).

Furthermore, BMI was also positively correlated with SBP and DBP, notwithstanding no significant correlation observed between FBG and the blood pressure markers. The observed significant positive correlations between BMI and the blood pressure markers are in agreement with previous studies (Gupta & Kapoor, 2010;Dua, Bhuker, Sharma, Dhall& Kapoor,2014). As reviewed by Dua*et al.*(2014), BMI is recognized as important predictor of CVD and hypertension, due to their positive association with hypertension. Meanwhile, relationships between BMI and other anthropometric indices have been established (Jimoh, Adediran, Agboola, Tomi-Olugbodi & Adebisi,2008;Marjani, 2011).

The present study also demonstrated significant negative correlations between serum GPx activity and TG and VLDL-c concentrations. Similar result was reported byRizman, Jalil & Zakaria(2012) suggesting that diabetic subjects who had reduction in their plasma TG and VLDL-cholesterol concentrations were associated with increased plasma GPx activity. This also indicates the coexistence of dyslipidaemia (increased TG and VLDL-c concentrations) and oxidative stress (decreasedGPx activity).

AC was the only anthropometric indices that related negatively to oxidative stress parameters (GSH concentration and CAT activity). There is need for more correlation studies between these parameters, since AC is a new alternative index proposed to measure obesity in type 2 diabetes (Devang*et al*, 2016).

The results also showed that TG, HDL-c and VLDL-c correlated negatively with CRI-I, CRI-II and AC (atherogenic coefficient). Similarly, HDL-c also correlated negatively with AIP. However, significant positive relationship existed between LDL-c and the atherogenicindices(except AIP), and between TC and nHDL-c alone. In a similar study,Ugwuja, Ogbonna, Nwibo, & Onimawo(2013) reported positive relationships between LDL-c and CRI 1 & 2, and negative correlation between HDL-c and CRI 1 & 2. Contrary to the results of the

present study, the report noted positive relationships between TC and CRI index 1 & 2 on one hand, and between TG and CRI-1 on another, but no correlation between TG and CRI-11 (Ugwuja *et al.*, 2013). The observed relationships between LDL-c, HDL-c and CRI index 1 & 2 are apparently logical, considering the fact that calculation of CRI-I(TC/HDL-c)and CRI-II (LDL-c /HDL-c) take into account both atherogenic and cardioprotective lipid fractions(Mohieldein*et al.*, 2014). This implies that as LDL-c (pro-atherogenic factor) increases and HDL-c(anti-atherogenic factor) decreases, the risk of atherogenicity increases.

It was observed in this study that HDL-c concentration related negatively withHt, HC, BMI, WHtR, SBP and DBP, while positive correlations were observed between the studied anthropometric indices and LDL-c.Negative correlations between HDL-c, as well as the positive correlation between LDL-c and TC, and various anthropometric indices observed in this studysuggests that anthropometric parameters could serve as ideal predictor indices fordylipidaemia, CVD risks, and hypertension in type 2 diabetics, considering the fact that decrease in HDL-c and increase in LDL-c and TC are strong indictors ofdyslipidaemia,and other diabetic complications, including hypertension. However, there isneed for further studies given the lack of consistency in the relationships so far reported between anthropometric and lipid profile indices in various studies. According to Himabindu*etal.*,(2013), positive correlations were observed only between BMI (> 25 kg/m^2) and VLDL in the entire diabetic subjects they studied, while inverse correlations were seen between WHR and HDL-c in type 2 diabetics without hypertension. A low strength negative correlation was reported between HDL-c, LDL-c and BMI in a recent study (Baltadjiev, Vladeva, Tineshev & Andreenko, 2016). In another study, WHRwas found to be significantly related to TG positively but negatively to HDL in male diabetics (Kanwar, Shekhawat, Rathore & Jain, 2016). In a study carried out among diabetic Koreans, WHR associated positively with TC and LDL-c in men, while inverse correlations were seen between WC and HDL in women (Sarah & Edwin, 2011). Further significant differences have been demonstrated in the relationships between anthropometric parameters and lipid profile patterns in type II diabetics in three different ethnic groups living in Malaysia (Blebil, Hassan & Dujaili,2011).

The results of the study showed that HC, BMI, WHtR, SBP and DBP were positivelycorrelated with nHDL-c, CRI-I, CRI-II and AC.CRI-I was reported previously to be weakly associated with both BMI and waist circumference (WC) in diabetic subjects (Diaf & Khaled, 2016). From our

results, AIP was positively correlated with BMI and WHtR. This observation is in agreement with two different studies which reported that AIP is positively related with WC, BMI, WHR and WHtR (Ezeukwu&Agwubike, 2014;Sharaye, 2015). The positive correlations observed in the present study between anthropometric indices, blood pressure and atherogenic indices suggest statistical reliability of these indices in the assessment of cardiovascular risks and hypertension among T2DM patients.

4.2.9 Dyslipidaemia distribution among the diabetic subjects

Results of our study showed that most of the diabetic subjects had their lipid levels deranged.Dyslipidaemia as a metabolic abnormality is frequently associated with diabetes mellitus. It has been shown to powerfully enhance the risk of developing atherosclerosis andhave been described as predictor of CVD including coronary heart diseases (CHD) in type 2 diabetes (Vergès, 2015; Nimmanapalli*et al.*, 2016). Our results revealed that 78.3%, 91.7%, 91.7% and 100% of the diabetics have abnormally high levels of TC, TG, VLDL-c and LDL-c respectively, while 35% of the subjects had low HDL-c. The results indicate thatthe sampled diabetic population may be predisposed to developing cardiovascular diseases in future. The observed 100% elevation in LDL-c of the diabetic subjects buttresses the recognition of LDL-c as 'bad cholesterol'. The elevation in LDL-c may be attributed partly to the effect of anti-lipid drugs used in thetreatment of complications in diabetes, which might have increased the activity of lipoprotein lipase (LPL)and hence thehydrolysisof VLDL (the precursor of LDL-c), thereby producing more LDL-c (Goldberg, 2001).

4.2.10 Abnormality inatherogenic indices of the diabetic subjects

This study showed that 71.6% of diabetic subjects had high nHDL-c.Non HDL-c is a measure of the amount of cholesterol carried by all lipoproteins except HDL. It is necessary to note that measuring total cholesterol alone or LDL alone provides limited information about CVD risk because the value for TC includes both HDL-c and LDL-c. Likewise, LDL is not the only lipoprotein involved in atherosclerotic heart disease. Non HDL-c is calculated by subtracting HDL-c from TC and therefore includes not only LDL-c but also cholesterol contained in all other apoB containing potentially atherogenic lipoproteins in blood, including cholesterol in

lipoprotein (a), intermediate density lipoprotein (IDL), very low density lipoprotein (VLDL) and cholesterol-enriched remnant lipoproteins (Sreevani *et al.,* 2016; Doc's Opinion, 2016). Studies have shown non-HDL-c to be analogous to Apo B in assessing atherogenic cholesterol and lipoprotein burden because of its high correlation with the apolipoprotein B levels (Hermans, Sacks, Ahn & Rousseau, 2011; Wei fen,Liang, Wang & fen FU, 2013). Moreover, the apolipoprotein B assay is not routinely available because of its cost and general unfamiliarity with its interpretation outside of the research setting (Nimmanapalli*et al.,* 2016).

Approximately 26.7%, 90% and 65%of the diabetic subjects showed abnormally high atherogenic levels of CRI-I, CRI-II and AC respectively. These indices are very useful in predicting cardiovascular risk as confirmed by a number of other studies (Nimmanapalli *et al.,* 2016; Bhardwaj *et al.,* 2013; Gordon *et al.,* 2010). Bhardwaj *et al.*(2013) concluded that lipid ratios like Castelli's risk indexesand AC could be used for identifying individuals at higher risk of cardiovascular disease, especially when the absolute values of individual lipoproteins seem normal and in individuals with elevated TG concentrations.

In the present study, 81.7% of the diabetic subjects had elevated levels of AIP. AIP is based on the ratio of the values of triacylglycerols to high-density lipoprotein cholesterol (HDL-c) calculated as log (TG/HDL-c). AIPvalues reflect the relationship of atherogenic lipids to protective lipids (Dobiášová *et al.,* 2011). Ahmed (2015) noted that AIP determinationdemonstrates cardiovascular risk in clinical trials.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The information gathered from the baseline characteristics of diabetic subjects showed that only 16.7% of the patients take vegetables daily, while 51.7% were on diabetic diet. Therefore adoption of a healthy dietary pattern such as daily consumption of starchy fiber foods, protein (nuts, eggs, tofu, beans, lentils, low-fat dairy products) and vegetables should be encouraged. Most of these are low in fat. As a result, this dietary pattern, apart from its various health benefits,including reduction of the risk of developing high levels of fats in the heart and blood vessels, is tasty and easy to practice in the long-term.

Some of the patients developed additional symptoms over time: feet numbness/leg pain (43.3%) chest pain (18.3%) and increased weakness (21.7%), which could be early signs of diabetes complications. Thus, the need for close monitoring and effective management of diabetic patients in order to maintain target levels for blood glucose, blood pressure, and cholesterol to reduce the risk of complications. Moreover, 63.3% of the subjects had BMI of $\geq 25 kg/m^2$, out of which 40% were obese. Weight loss through regular exercise and proper diet is recommended, which will aid the patients to respond to insulin better, maintain normal cholesterol levels, control blood glucose level and prevent CVDs.

The comparison between the diabetic and non-diabetic subjects showed significant elevated concentrations of TC, TG, LDL-c and VLDL-c but lower HDL-c in the diabetic subjects. There was observed high prevalence of dyslipidaemiaas most of the patients had their lipid profile deranged. These results, coupled with high values of atherogenic indices in the diabetic subjects, imply that type2 diabetic subjects might be at increased risk of developing atherosclerosis. Therefore, lipid profiling of all patients with type 2 diabetes mellitus should be a routine test. Theuse of lipid-lowering drugs should go hand-in-hand with antidiabetic drugs to reduce the risk ofatherosclerosisand coronary artery diseases (CAD).Furthermore, because atherogenic indices involve simple calculations, and are easily available with every lipid profile ordered without any additional costs, they are recommended to complement the existing lipid profile tests for screening and identifying high risk individuals for CAD and effective drug management.

Decreased efficiency of cellular antioxidant mechanisms in diabetic subjects (indicated by reduced concentration of serum GSH and reduced activities of antioxidant enzymes-SOD, GPx and CAT) with simultaneous enhanced lipid peroxidation (increased level of MDA) were observed in this study. These strongly suggest the involvement of oxidative stress in the pathophysiology and progression of diabetes in these patients. Hence therapies and life style changes having capacity to reduce the impact of oxidative stress may be beneficial in the management of the diabetic subjects.

MDA and GSH appear to be reliably deranged markers in hyperglycaemia since FBG significantly correlated positively with MDA but negatively with GSH.

No positive correlation was found between FBG and anthropometric indices, suggesting that anthropometric measurements may not be useful in predicting hyperglycaemia in diabetic patients. However, BMI may be important predictor of hypertension in diabetics due toa significant positive correlation observed between BMI and both systolic and diastolic blood pressures.

Negative correlations observed between HDL-c, as well as the positive correlations between LDL-c, TC and various anthropometric indices observed in this studyimplies that anthropometric parameters may be ideal for predicting dylipidaemia, CVD risks, and hypertension in the type 2 diabetics.

Among all the atherogenic indices, onlynHDL-c was found to be positively and significantly correlated with FBG of the subjects, suggesting that nHDL-c may be a better predictor of hyperglycaemia and in extension CVD risk than a simple measurement of TC, TG, HDL-c or LDL-c in type 2 diabetes mellitus subjects.Significant negative relationship between HDL-c and all the atherogenic indices, as well as positive correlations between LDL-c and the atherogenic indices, and between TC and nHDL-c, showed that atherogenic indices could be used as alternative indices for identifying individuals at high risk of cardiovascular disease in clinical practice, especially when the absolute values of lipid profile are not markedly deranged. Moreover, they involve simple calculations, and are easily available with every lipid profile ordered without any additional costs.

The positive correlation observed in this study between atherogenic indices, blood pressure and anthropometric indices suggests statistical reliability of these indices in assessment of cardiovascular risks and hypertension.

In conclusion, the results of the study have shown that determination of lipid profile and oxidative stress parameters of diabetic patients and a study of their correlationswithatherogenicand anthropometric indices could provide useful markers for the diagnosis and /or prognosis of diabetes. Detection of the risk factors using these markers in early stages of the disease will help to reduce diabetic complications and mortality rate.

5.2 Recommendations

Results of the present study make the under listed recommendations pertinent.

1. More extensive studies are needed to establish the basis for gender differences observed inbaseline information and atherogenic indices which may be of clinical significance.
2. Females had significantly higher BMI and WC than males in both diabetics and non-diabetics in this study, which is in consonance with previous reports. This buttresses the need to determine the possible contributing factors and probably assign separate BMI and WC cut-off points for gender groups.
3. There is need for more correlation studies between anthropometric indices and oxidative stress parameters. This is necessary to, among others, explainthe basis for the observed negative relationship betweenarm circumference (AC) and oxidative stress parameters (GSH concentration and CAT activity).
4. Consistency between anthropometry and lipid profile indices needs to be determined by further extensive correlation studies.

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APPENDIX

1: SUMMARY TABLES

Glucose concentration and lipid profile parameters of diabetic and non-diabetic subjects

Parameters	All groups		Male		Female	
	Control (n = 40)	Test (n=60)	Control (n=20)	Test (n=30)	Control (n=20)	Test (n=30)
Glucose (mg/dl)	93.87 ± 15.33	176.42 ± 46.86	96.05 ± 16.61	171.33 ± 43.78	91.70 ± 14.02	181.50 ± 49.97
TC (mmol/L)	3.55 ± 0.89	5.68 ± 0.66	3.17 ± 0.85	5.81 ± 0.62	3.92 ± 0.77	5.55 ± 0.68
TG (mmol/L)	1.03 ± 0.41	2.63 ± 0.71	0.97 ± 0.42	2.59 ± 0.69	1.09 ± 0.40	2.67 ± 0.75
HDL-c (mmol/L)	1.61 ± 0.26	1.20 ± 0.40	1.62 ± 0.28	1.02 ± 0.32	1.60 ± 0.24	1.38 ± 0.40
VLDL-c (mmol/L)	0.47 ± 0.19	1.20 ± 0.32	0.44 ± 0.19	1.18 ± 0.31	0.49 ± 0.18	1.21 ± 0.34
LDL-c (mmol/L)	2.40 ± 0.82	5.68 ± 0.74	2.00 ± 0.66	5.96 ± 0.68	2.81 ± 0.76	5.39 ± 0.69

Atherogenic indices of diabetic and non-diabetic subjects

Parameters	All groups		Male		Female	
	Control (n = 40)	Test (n=60)	Control	Test	Control	Test
nHDL-c (mmol/L)	1.95 ± 0.83	4.48 ± 0.79	1.63 ± 0.79	4.78 ± 0.50	2.27 ± 0.76	4.18 ± 0.92
CRI -I	2.27 ± 0.70	5.31 ± 1.19	2.04 ± 0.70	6.15 ± 1.75	2.50 ± 0.63	4.47 ± 1.72
CRI-II	1.55 ± 0.63	5.29 ± 2.07	1.30 ± 0.53	6.19 ± 1.90	1.80 ± 0.62	4.40 ± 1.87
AC	1.27 ± 0.63	4.31 ± 1.91	1.07 ± 0.58	5.15 ± 1.75	1.46 ± 0.62	3.47 ± 1.72
AIP	-0.21 ± 0.16	0.33 ± 0.11	-0.25 ± 0.20	0.36 ± 0.09	-0.19 ± 0.11	0.31 ± 0.12

Oxidative stress parameters of diabetic and non-diabetic subjects

Parameters	All groups		Male		Female	
	Control (n = 40)	Test (n=60)	Control	Test	Control	Test
GSH(µmol/L).	4.40 ± 0.88	3.05 ± 0.78	4.46 ± 0.92	3.02 ± 0.88	4.35 ± 0.87	3.08 ± 0.67
GPx(U/ml)	0.78 ± 0.12	0.58 ± 0.06	0.79 ± 0.11	0.58 ± 0.05	0.77 ± 0.13	0.58 ± 0.06
SOD(U/ml)	18.94 ± 1.47	12.85 ± 2.16	19.22 ± 1.42	13.25 ± 2.27	18.66 ± 1.51	12.45 ± 2.02
CAT(kU/l)	58.52 ± 8.68	40.97 ± 7.50	58.97 ± 7.40	38.59 ± 8.32	58.06 ± 9.96	43.35 ± 5.79
MDA (nmol/ml)	1.36 ± 0.26	2.75 ± 0.58	1.31 ± 0.18	2.65 ± 0.56	1.41 ± 0.33	2.84 ± 0.59

Anthropometric indices and blood pressures of diabetic and non-diabetic subjects

Parameters	All groups		Male		Female	
	Control (n = 40)	Test (n=60)	Control	Test	Control	Test
BMI (kg/m^2)	20.98 ± 2.92	27.43 ± 5.64	20.30 ± 3.41	24.57 ± 4.16	21.66 ± 2.22	30.30 ± 5.51
WC (m)	0.84 ± 0.07	0.99 ± 0.08	0.79 ± 0.07	0.96 ± 0.08	0.89 ± 0.04	1.02 ± 0.08
HC (m)	1.00 ± 0.10	1.02 ± 0.10	1.04 ± 0.09	0.97 ± 0.07	0.97 ± 0.10	1.07 ± 0.11
AC (m)	0.31 ± 0.04	0.35 ± 0.03	0.29 ± 0.03	0.35 ± 0.03	0.33 ± 0.03	0.35 ± 0.03
WHR	0.84 ± 0.10	0.98 ± 0.09	0.86 ± 0.07	0.99 ± 0.07	0.82 ± 0.12	0.96 ± 0.10
WHtR	0.50 ± 0.04	0.61 ± 0.07	0.51 ± 0.04	0.56 ± 0.05	0.49 ± 0.03	0.66 ± 0.06
SBP (mmHg)	125.50 ± 14.22	140.92 ± 11.81	128.50 ±14.88	138.67 ± 10.42	122.50 ± 13.23	143.17 ± 12.83
DBP (mmHg)	78.50 ± 8.34	82.33 ± 6.67	80.00 ±8.58	80.33 ± 7.18	77.00 ± 8.01	84.33 ± 5.53

2: QUESTIONNAIRE FOR RESEARCH

Dear Respondent,

This questionnaire is designed to obtain information that would help improve the management of diabetic patients, kindly answer the questions truthfully to the best of your knowledge and be assured of strict anonymity and confidentiality of your information. Thank you for your co-operation.

Please tick as appropriate

BIODATA

1. Sex: Female□ Male□

2. Age: 40-44□ 45-49□ 50-54□ 55-59□ 60-64□ 65-70□

3. Marital status single□ Married□ divorced□ separated□ cohabiting □

4. No of children_____ Nationality Nigerian □ others specify_____

5. State of origin_____ 6.Residential town_____

7. Educational level, No formal education□ Primary□ Secondary□ Diploma or equivalent□ University□

8. Employment status, Employed□ Unemployed□

9. Kind of employment_____

NUTRITION

10. Favourite meals (4 bests)_____ _____

_____ _____

11. Do you like vegetables? Yes□ No□ 12 .Types of vegetables consumed, Green vegetable□ carrot and cabbage□ cucumber□ Garlic and onions□ Okro□. Others specify_____

13. How often do you take these vegetables? Once Weekly□ Everyday□ Thrice Weekly□ Others Specify_____

14. Do you like fruits? Yes□ No□ 15. How often do you take the fruits? At every meal □ Once Daily □ Twice Daily □ Twice Weekly □ Others Specify_____

16. Number of full meals taken daily. More than 3□ 3times□ 2times□ 1time□

17. Have you ever smoked? Yes□ No□ 18. Are you still smoking? Yes□ No□

19. If no, when did you stop? _____

20. Do you drink alcohol? Yes□ No□ 21.How many bottles a day_____

22. Have you ever used any hard drugs? Yes□ No□ 23. Which ones? Ordinary cigar□ Indian hemp□ heroine□ cocaine□, others specify_____

24. Do you use these drugs by injection? Yes□ No□

PAST MEDICAL HISTORY

25. Do you experience (i) increased thirst and hunger? Yes□ No□, weakness/fatigue? Yes□ No□ (ii) Blurred vision Yes□ No□ (iii) Feet numbness Yes□ No□ (iv) frequent urination Yes□ No□.

26. Are you diabetic? Yes□ No□ 27.How long have you been diabetic_____

28. Are you on any diabetic drug(s)? Yes□ No□ Which one(s)

_____

29. Treatment duration_____ Do you feel you are improving? Yes□ No□

30. Do you have high blood pressure? Yes□ No□Blood pressure _____

31. Do you take multi-vitamins or any vitamin drug(s)? Yes□ No□, which one(s)?_____

32. What other symptoms are you experiencing different from the earlier ones?_____

33. Have any of the members of your immediate family or other relatives been diagnosed with diabetes? Yes□ No□,

if yes, who? grandparent aunt□ uncle□ first cousin□ ; parent□ brother□ sister□ own child□

34. How often do you check your blood glucose level? Once a week□ twice a week□

Once in two weeks□, others specify_____

35. The level increases□ decreases□ fluctuate

ANTHROPOMETRIC DATA

36. Height_____ 37. Weight_____

38. Waist Circumference_____ 39. Hip Circumference _____

40. Arm Circumference _____

3: ETHICAL CLEARANCE

CHUKWUEMEKA ODUMEGWU OJUKWU UNIVERSITY TEACHING HOSPITAL, AMAKU AWKA

(Formerly Anambra State University Teaching Hospital)

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Chioma Ibekie B.Sc
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Chief Medical Director

Dr. Ifeanyi Ezeobi MBBS, FWACS
Chairman Medical Advisory Committee (CMAC)

Dr. Ejiofor O.S MBBS, FWACP (paed)
Deputy-CMAC

Our Ref: COOUTH/AA/VOI.I.012 ***Date: 31st May, 2016***

ETHICAL CLEARANCE

TOPIC: Oxidative stress and Dyslipidaemia in type-2 diabetic patient attending Chukwuemeka Odumegwu Ojukwu University Teaching Hospital Awka

BY: Nwankwo Chinenye

FOR: Award of MSc in the Department of Biochemistry, Federal University of Technology Owerri

The above named research topic has been reviewed and approved by the Ethical Committee of COOUTH Amaku, Awka.

Yours faithfully,

DR. EJIOFOR O.S. (FWACP)
Chairman Ethical Committee