

**EVALUATION OF SERUM TOTAL ANTIOXIDANT
STATUS IN TYPE II DIABETIC NIGERIANS**

BY

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AHMADU BELLO UNIVERSITY, ZARIA, IN PARTIAL
FULLFILMENT OF THE REQUIREMENTS FOR THE
AWARD OF MASTERS DEGREE
IN CHEMICAL PATHOLOGY.**

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DECLARATION

I hereby declare that the work reported in this thesis entitled “ Evaluation of serum total antioxidant status in type II diabetic Nigerians” was performed by me in the Department of Chemical Pathology, Faculty of Medicine of Ahmadu Bello University, Zaria. The information derived from the literature was duly acknowledged in the text and a list of reference provided. No part of this thesis was previously presented for another degree or diploma in any university.

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DEDICATION

This work is dedicated to the Almighty God, to my Parents, Mr and Mrs Yakubu Akawu Yohana.

ACKNOWLEDGEMENT

I thank the Almighty Father for His loving kindness, grace and mercy, for making this dream come true. I return to you the glory. I would not have done this on my own without you. To my team of supervisors, Prof. PO Anaja, Dr SA Akuyam and Dr AG Bakari, I say a very big thank you for the patience, encouragement and constructive criticism towards the success of this work. You are wonderful people. The same gesture goes to Dr IS Aliyu, my lecturer for his great concern and encouragements throughout the period of this study. I also thank Prof HS Isah for his great concern. Dr SA Luka of Biological Sciences Department and Dr E. E. Ella of Biotechnology center, both of Ahmadu Bello University, Zaria. Thanks to Mr Ladan and Mr Gandu, both of National Research Institute of Chemical Technology, Zaria.

A big thank you to Mr Ekanem the departmental technical head and the entire staff and students who assisted me in one way or the other. God bless you all. Special thanks to Mal. MA Bello, Mal. N Lawal, Dr Y Rasheed, Dr IA Isa and Mr Idowu. I thank you all for your great support.

My sincere gratitude goes to my parents Mr and Mrs Yakubu A Yohana for their great support and encouragements, also to my husband, children, brothers sisters, Mr Abdulkarim Luka and Mr Galadima Abare. I thank all my friends for their prayers and encouragement. I appreciate you all. God bless you.

ABSTRACT

Patients with type II diabetes mellitus are more prone to diabetic complications and oxygen free radicals are known to contribute to the development of complications but there are conflicting reports regarding antioxidant status in type II diabetic patients. The current cross sectional study was designed to evaluate the serum total antioxidant status (TAS) in type II diabetic patients and age matched control subjects in ABUTH Shika, Zaria. Also to assess their correlation with clinical parameters and biochemical analytes. A total of 281 subjects were recruited for the study. These comprised of 181 type II diabetic patients and 100 controls. Fasting blood glucose (FBG) was measured using glucose oxidase method, glycated haemoglobin (GHbA_{1c}) using micro column method, TAS using Mosmann method while vitamins C and E using Moeslinger and Dahot methods respectively. The reference values of TAS, vitamins C and E are 15.7 – 63.1 mmol/L, 0.2 – 7.0 mg/ml and 3.7- 119.3 g/L respectively. The mean value of TAS (33.3 ± 0.5 mmol/L) was significantly lower ($p < 0.05$) in diabetic patients than in control subjects (39.4 ± 0.8 mmol/L). While on the other hand the mean values of FBG and GHbA_{1c} in diabetic patients (7.0 ± 0.3 and 8.2 ± 0.2) were significantly higher ($p < 0.05$) than corresponding values in controls (4.1 ± 0.8 and 5.0 ± 0.1) respectively. The mean values of TAS were however similar ($p > 0.05$) among diabetic patients with good and poor glucose control (33.3 ± 0.7 mmol/L versus 32.2 ± 0.7 mmol/L). Also the mean values of TAS were similar ($p > 0.05$) among diabetic patients with good and poor GHbA_{1c} control (33.4 ± 0.6 mmol/L versus 33.1 ± 0.6 mmol/L). Similarly, the mean values of TAS in diabetic patients with complications and those without complications (32.8 ± 0.6 mmol/L versus 34.4 ± 1.1 mmol/L) were not significantly different ($p > 0.05$). The mean value of vitamin E was found to be significantly higher ($p < 0.05$) in patients with good glucose control (44.6 ± 3.0 g/L) and glycated haemoglobin control (44.5 ± 2.7 g/L) than in those with poor glucose control (41.8 ± 2.7 g/L) and glycated haemoglobin control (41.7 ± 2.8 g/L). Furthermore, the mean level of vitamin E in patients without complications (49.5 ± 7.0 g/L) was significantly higher ($p < 0.05$) than in those with complications (42.5 ± 2.1 g/L). In diabetic patients, there were positive and significant correlations between TAS and age ($r=0.173$, $p < 0.05$) as well as duration of diabetes mellitus ($r=0.240$, $p < 0.05$). These results suggest that type II diabetic patients of the study area have low serum level of TAS.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

Diabetes mellitus (DM) is a disorder of carbohydrate metabolism, whereby the body is not able to properly utilize glucose (Papap, 2008). Rahboni- Nobar *et al* (1999) stated that DM is a syndrome that is characterized by chronic hyperglycaemia which is due to dynamic interactions between varying defects of insulin secretion and resistance. DM is the commonest endocrine disorder seen in clinical practice with high morbidity and mortality rates (Bakari *et al*, 2003).

Diabetes mellitus is an epidemic disease in most countries that are undergoing socio-economic transitions (Rahboni- Nobar *et al*, 1999). Worldwide, an estimated 150 million people are affected by DM and this number is likely to reach 300 million by the year 2025 resulting in approximately 450,000 deaths a year, if successful strategies for its prevention and control are not implemented (Rahboni- Nobar *et al*, 1999; WHO, 2000; Papas, 2008).

The prevalence of DM varies from country to country and from one community to the other. According to the 2004/05 Australian National survey, approximately 3 % of the population were reported to be diabetic (Papap, 2008). In Nigeria, the national prevalence of diabetes mellitus is

estimated to be 2.7 % while the northern part of Nigeria has a prevalence of 1.6 % (Bakari *et al*, 1999).

Diabetes mellitus is classified into two major classes. The less common class is type I (formerly known as insulin – dependent diabetes mellitus, IDDM) and the most common class is type II (formerly known as non – insulin dependent diabetes mellitus, NIDDM). Other specific types of DM include; genetic defects of β -cell function, genetic defects in insulin action, endocrinopathies, infection induced, drug, chemicals and gestational DM (Papas, 2008). This classification was endorsed by WHO expert committee on diabetes in 2000 (WHO, 2000). Measurement of post challenge (oral glucose tolerance testing) was previously the gold standard for the diagnosis of DM. More recently, the American Diabetes Association (ADA) has advocated the use of fasting plasma glucose (FPG) as an equally useful diagnostic test, but postprandial plasma glucose (PPG) remains more sensitive and specific marker of glucose intolerance (Kendall, 2005).

Hyperglycaemia results in a cascade of events leading to different complications and vascular damages (Kaneto *et al*, 1999; Gupta and Chari, 2006; Lai, 2008; Papas, 2008). These complications could be microvascular or macrovascular, but long term complications are the main causes of morbidity and mortality (Rahbani – Nobar *et al*,1999; Lai,

2008). Report shows that in Taiwan, there has been a five- fold increase in mortality from DM –related complications for the last 20 years (Lai, 2008). Recent studies show that for type II DM, there is high incidence of oxidative complications, such as retinopathies, glomerulopathies and vascular complications (Lai, 2008).

Human life is dependent on oxygen because it oxidizes food and generates energy (Richard, 1997). Oxidant/free radicals are species with very short half life, high reactivity and damaging activity towards macromolecules like proteins, de-oxyribonucleic acid (DNA) and lipids (Kaneto *et al*, 1999; Ceriello, 2004; Papas, 2008; Tiganis *et al*,2009). These species may either be oxygen derived (ROS - reactive oxygen species) or nitrogen derived (RNS – reactive nitrogen species). The oxygen derived species include hydroxyl (OH), hydroperoxyl (HO₂), peroxy (ROO), alkoxy (RO) as free radicals and hydrogen peroxide (H₂O₂), hypochloride (HOCl), ozone (O₃) and single oxygen (O₂) as non-radicals. RNS are mainly nitric oxide (NO), peroxynitrite (ONOO), nitrogen dioxide (NO₂) and di-nitrogen trioxide (N₂O₃) (Irshad and Chaudhuri, 2002; Ceriello, 2004).

Antioxidants are protective proteins, vitamins, minerals and enzymes because they fight oxidative stress by preventing cell damage caused by charged particles. These charged particles are known as reactive oxygen species. This oxidative stress is thought to add to the

progression of several diseases including type II DM (Nuttall *et al*,1998; Kaneto *et al*,1999). Reactive oxygen species (ROS) are disproportionately formed in diabetics which may eventually lead to complications of the disease (Kaneto *et al*,1999). In DM during glucose metabolism, the body cells are going through oxidative stress, where abnormally high levels of free radicals are produced in the system which can cause cell damage thus decreasing the number of cells (Kaneto *et al*, 1999; Rahbani – Nobar *et al*,1999; Gupta and Chari, 2006; Papas, 2008).

Every second, tens of thousands of free radicals are created in the body (Richard, 1997). These free radicals lead to an increase in oxidizing response above a certain threshold which in the absence of concomitant rise in antioxidant/reducing response, leads to oxidative stress which is associated with the complications of DM (Medina *et al*, 2007). The prime targets of these radical reactions are the unsaturated fatty acids which have a role to play in membrane fluidity, receptor alignment and when compromised cellular lyses follows. Damage to carbohydrate can alter any of the cellular receptor functions including those associated with hormonal neurotransmitter responses (Sies, 1995; Gupta and Chari, 2006).

It has been suggested that overproduction of reactive oxygen and nitrogen species may be involved in the initiation and development of vascular complications in DM (Rahbani – Nobar *et al*,1999; Kaneto *et al*,

1999; Irshad and Chaudhuri, 2002). Epidemiological studies revealed that there is indisputable evidence from a shift in the equilibrium between ROS and antioxidants in favour of oxidative stress in DM (Baynes, 1991; Amstrong *et al*, 1992; Kowluru *et al*, 1997; Kowluru *et al*, 2000). These abnormalities can be seen in pre-diabetic state and therefore early intervention can be beneficial (Balasubramanyam *et al*, 2005).

Oxidative stress has been proposed as a unifying hypothesis linking various molecular disorders of DM. The theoretical importance of oxidative stress in DM is highlighted by the potential double impact on metabolic abnormalities and on the vascular system (Balasubramanyam *et al*, 2005). The potential contribution of increased oxidative stress to the development of complications of DM is of growing interest (Alberti *et al*, 1997). Metabolic stress arising from changes in energy metabolism, alteration in sorbitol pathway activity, changes in the levels of inflammatory mediators and the status of antioxidant defense systems all contribute to the oxidative state in DM (Baynes, 1991).

Oxidative stress is very common in diabetic patients and this stress is known to induce damages to both plasma components and cells but the human blood plasma is considered to be well equipped with preventive antioxidant effectors (Medina *et al*, 2007). Vitamins C and E are naturally occurring antioxidants that prevents damages such as lipid peroxidation

and these vitamins, uric acid and glutathione are all found to be decreased in diabetics (James , 1990; Gupta and Chari, 2006).

Increased levels of antioxidants in the system may be of great advantage to diabetic patients because protein glycosylation could be reduced and glucose autooxidation and formation of advanced glycated end products (AGEs) could also be inhibited (Alberti *et al*, 1997). These observations led a number of researchers to use different agents with antioxidant effect in diabetic patients so as to reduce the oxidative stress. These authors show that antioxidant vitamins and mineral supplementation led to the reduction of peroxidative development (Opara *et al*, 1999). Murugeson *et al* (2005) confirmed the protective effect of vitamins C and E in scavenging the oxidative effect of co- administration of Aroclor 1254 (an oxidant) in leydig cells of diabetic rats.

Vitamins can donate as well as accept electrons hence quenching free radicals, while minerals regulate the activity of the antioxidant enzymes (Opara *et al*, 1999). Protective effects of vitamins C and E as well as glutathione in plasma have also been reported (Nwanjo *et al*,2007). Many researchers supported the need for increase in antioxidant levels in the body so as to minimize the deleterious effects of free radicals generation in DM, since antioxidants are meant to “mop up” free radicals in the body (Ceriello, 2004; Gupta and Chari, 2006; Medina *et al*, 2007; Papas, 2008). Opara (2002) recommends high doses of micronutrient

antioxidants which interact with each other in a biochemical chain of defense.

1.2 STATEMENT OF PROBLEMS

Diabetes mellitus leads to many disease complications from long term oxidative damage to tissues by free radicals arising from glucose metabolism which later results in increased morbidity and mortality (Gupta and Chari, 2006). Once these complications occur it may be irreversible and meeting the cost of management of complications is a challenge which will increasingly confront diabetic patients and health facilities in developing countries (Baynes 1991).

Patients in developing countries like Nigeria where health care is not free may be faced with what is referred to as intolerable burden. The most important questions for clinicians caring for diabetic patients, and for the patients themselves is weather the risk of complications can be eliminated or minimized and hospital admission reduced (Irshad and Chaudhuri, 2002).

It has also been reported that the role of antioxidant vitamins in the therapy of DM is of significant importance (Ernahr, 1994). In diabetic patients, an altered balance between ROS production and antioxidant levels has been reported (Rahbani – Nobar *et al*,1999). ROS activates complements (C₄ and C₅) which leads to cell binding. A correlation between infiltrative damage and decreased plasma antioxidant status has been also suggested (Medina *et al*, 2007). Oxidative stress and associated tissue damage represent a common end point of chronic diseases such as

DM (Medina *et al*, 2007). Hence there is need to evaluate the levels of total antioxidant status (TAS) in DM.

A study shows that good blood glucose control can reduce the risk of heart disease and stroke among diabetic patients by 15 % (Ians, 2009). It has also been reported that patients with type II DM are more prone to ischaemic heart disease than those with other types of diabetes mellitus (Gupta and Chari, 2006). A study in Iran shows that there is a significant correlation between total antioxidant status (TAS) and poor glycaemic control, hence the suggestion that measurement of TAS in DM could be used as an index of glycaemic control and development of diabetic complications (Rahbani – Nobar *et al*,1999).

There is paucity of data on the levels of TAS in type II diabetic patients in our environment. Most of the studies were carried out in other parts of the world (Aliyu *et al*, 2005; Murugeson *et al*, 2005; Gupta and Chari, 2006; Afkharni-Ardekan and Shojaodding-Ardekani, 2007) hence, there is need for further studies to evaluate the levels of these antioxidants in diabetic patients owing to their numerous advantages in preventing diabetic complications.

1.3 OBJECTIVES OF THE STUDY

1.3.1 *General Objective*

The general objective of the study was to evaluate the serum total antioxidant status in type II diabetic patients.

1.3.2 *Specific Objectives:*

The specific objectives of the present study:

1. **To measure serum TAS.**
2. **vitamins C and E, glucose and glycated haemoglobin levels in type II diabetic patients and controls.**
3. **To compare the values of serum TAS, vitamins C and E, glucose and glycated haemoglobin in diabetic patients and controls.**
4. **To assess the effect of glycaemic control on TAS in diabetic patients.**
5. **To assess the relationship if (any) between long term complications of DM and TAS in diabetic patients.**
6. **To compare the results of clinical and biochemical parameters obtained in diabetic patients and controls.**

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 DIABETES MELLITUS

Diabetes mellitus is a chronic disease characterized by high levels of glucose resulting from defects in insulin production, insulin action or both (Aliyu *et al*, 2005). DM is a multisystem disease that is wide spread throughout the world affecting carbohydrate, protein and lipid metabolisms (Aliyu *et al*, 2005). Abnormally high glucose levels can be harmful to individual's health and can lead to serious complications. A number of studies have suggested that enhanced oxidation is the underlying abnormality responsible for some of the complications of DM (Hassan and Mooradian, 2007).

2.1.1 Classification of Diabetes Mellitus

Diabetes mellitus is classified into two major groups. In 1979, a working group established by the United States of America, the National Diabetes Data Group (NDDG) recognised two major forms of DM and they were termed insulin-dependent diabetes mellitus (IDDM) and non-insulin dependent diabetes mellitus (NIDDM). This classification was later endorsed by WHO expert committee on diabetes (WHO, 2000). Other specific types of DM were also recognized. They include Gestational Diabetes Mellitus (GDM), impaired glucose tolerance (IGT) and impaired fasting glucose (IFG).

In addition, in 1995 the American Diabetes Association (ADA) established a working group to re-examine the classification and diagnosis of DM. The revised classification, published in 1997 by ADA, eliminates the terms insulin-dependent diabetes mellitus and non-insulin dependent diabetes mellitus, which are now termed type I and type II diabetes mellitus respectively. This revised classification is shown in table 2.1

2.1.1.1 Type 1 Diabetes Mellitus

Type I diabetes mellitus (Type I DM) was also called insulin dependent diabetes mellitus (IDDM) or juvenile onset diabetes mellitus (Deets, 2004). The pancreas here undergoes an autoimmune attack by the body itself and is rendered incapable of producing insulin. Because of these, type I diabetic patients must take daily doses of insulin for survival. Abnormal antibodies are produced in type I DM and it is believed that the tendency to develop abnormal antibodies in type I DM is in part genetically whetted. Type I DM occurs in 5-10 % of diagnosed DM cases in North America, Europe and Nigeria (Greenfield *et al*, 2002; Bello-Sani *et al*, 2007). Most often, type I DM develops in young children and adults although, disease unset can occur at any time (Deets, 2004).

2.1.1.2 Type II Diabetes Mellitus

Type II diabetes mellitus (type II DM) is characterized as a polygenic disorder generally thought of as a syndrome rather than a single specific entity (Robertson *et al*, 2003). Type II DM was referred to as non-insulin dependent diabetes mellitus (NIDDM) or adult onset diabetes mellitus (AODM). Type II DM is the most common type and it is found in 90-95 % of diagnosed DM cases (Aliyu *et al*, 2003). The major feature of type II DM is a lack of sensitivity by the cells of the body to insulin at early stage, which is characterized by elevated levels of insulin in the blood (Robertson *et al*, 2003; Aliyu *et al*, 2005). At early stage, hyperglycaemia can be reversed by a variety of measures and medications that can improve insulin sensitivity or reduce the rate at which the liver produces glucose.

Table 2.1: Classification of diabetes mellitus

<p>I. type I diabetes mellitus</p>	<p>a. Immune mediated</p> <p>b. Idiopathic</p>
<p>II . Type II diabetes mellitus.</p>	
<p>III. Gestational diabetes mellitus</p>	
<p>IV. Other specific types</p>	<p>a. Genetic defects of β-cell function</p> <p>b. Genetic defects of insulin action</p> <p>c. Diseases of the exocrine pancreas</p> <p>d. Endocrinopathies</p> <p>e. Drug or chemical induced</p> <p>f. Infections induced</p> <p>g. Uncommon forms of immune mediated diabetes</p> <p>h. other genetic syndromes sometimes associated with diabetes.</p>

ADANACB, 2002.

As DM progresses the impairment of insulin secretion worsens, and therapeutic replacement of insulin often becomes necessary (Borette *et al*, 2002). It was reported that type II DM occurs mostly in individuals over 30 years old and the incidence increases with age (Ruhe and McDonad, 2003). Studies show that 20 % of elderly patients in North America having DM, their family history revealed that type II DM is much more common in those with close relatives who have it (Greenfield *et al*, 2002). Presently the condition is alarming because a number of type II diabetic patients are in their teenage years. Studies show that type II DM is more common in children than type I (Ruhe and McDonald, 2003; Deets, 2004). Most of these cases are as a result of poor eating habits, higher body weight and lack of exercise (Deets, 2004).

2.1.1.3 Gestational Diabetes Mellitus

This form of DM occurs temporarily during pregnancy. Significant hormonal changes during pregnancy can lead to blood glucose elevation in genetically predisposed individuals (Abayomi, 2003). Blood glucose elevation during pregnancy is called gestational DM and this usually resolves once the baby is born. Worldwide, gestational DM is present in about 4 % of all pregnancies or about 135,000 annually with prevalence

range of 1-14 % depending on population studied and diagnostic test employed (Abayomi, 2003).

In Nigeria, the incidence rate of diabetic pregnancy (gestational and pre-gestational) was reported as 1 in 1000 per year (Abayomi 2003). David (2000) reported that gestational DM is usually asymptomatic and not life-threatening to the mother. However, it is associated with increased incidence of neonatal morbidity including hypocalcaemia, hypoglycaemia and macrosomia.

Some women (25-50 %) with gestational DM are likely to develop type II DM later in life especially those who require insulin during pregnancy and those who remain overweight after delivery. Women with gestational DM should undergo oral glucose tolerance test (OGTT) six weeks after delivery to see if their DM has persisted beyond the pregnancy.

2.1.1.4 Other Specific Forms of DM

These other forms of DM are characterized by elevated blood glucose level from other medical conditions. This can occur when there is destruction of pancreatic tissue by diseases such as chronic pancreatitis (inflammation of the pancreas by toxins like excessive alcohol), trauma or surgical removal of the pancreas.

Diabetes mellitus can also result from other hormonal disturbances such as Acromegaly and Cushing's syndrome. Some cases of DM are

caused by receptors not responding to insulin although insulin levels are normal, which is very uncommon (Sacks *et al*, 2002).

2.1.2 Risk Factors for Developing Type II DM

Apart from the strong genetic component for developing type II DM, there are other risk factors. The most significant risk factor is obesity. Studies show that there is a direct relationship between the degree of obesity and the risk of developing type II DM (Ruhe and McDonald, 2003). Estimate shows that the chance to develop DM doubles for every 20 % increase over desirable body weight (Ruhe and McDonald, 2003). In order to prevent and treat type II DM a combination of weight reduction and exercise has to be observed. Studies show that 65-75 % of cases of DM in Caucasians could be avoided if individuals in this subgroup did not exceed their weight (Ruhe and McDonald, 2001). Research also shows that skeletal muscle responds to exercise training by increasing the concentration of glucose transported thereby resulting in improved insulin action (Ruhe and McDonald, 2001).

Age is also a significant risk factor for developing type II DM (Nuttall *et al*, 1998). Study shows that for each decade after 40 years of age regardless of weight there is always an increase in incidence of DM (Nuttall *et al*, 1998; Ruhe and McDonald, 2003). The prevalence of DM in person of 65-74 years of age is nearly 20 % (Aliyu *et al*, 2005). Women

with a prior history of DM that develop during pregnancy are also at risk of developing type II DM (Ruhe and McDonald, 2003).

2.1.3 Causes of Diabetes Mellitus

Diabetes mellitus is caused by insulin insufficiency either absolutely or relatively to the body's need. Production of defective insulin or inability of cells to use insulin properly and efficiently lead to hyperglycaemia and DM. This later affects mostly the cells of muscles and fat tissues leading to insulin resistance. This is a primary problem in type II DM. The absolute lack of insulin usually secondary to a destructive process affecting the insulin producing β - cells of the pancreas is the main disorder of type I diabetes mellitus. In type II diabetes mellitus, there is a steady decline of β cells that adds to the process of elevated blood glucose (Robertson *et al*, 2003; Renier, 2007).

2.1.3.1 *Glucose and Insulin*

Glucose is a simple sugar and it is a nutrient that provides energy for the proper functioning of the body cells. Glucose in the blood stream cannot enter the cells of the muscles alone, it needs insulin to aid its transport into the cells. Without insulin the cells become starved of glucose despite the presence of abundant glucose in the system. These glucose is then wastefully excreted in the urine.

The hormone insulin is produced by the pancreas specialized β - cells. It is important in tightly regulating the level of glucose in the blood.

When there is hyperglycaemia, more insulin is secreted into blood stream so as to keep the glucose level in a tight controlled range (Renier, 2007). In patients with DM, the insulin is either absent, relatively insufficient for the body's need or not properly used. All of these cause elevated levels of glucose (Renier, 2007). When insulin resistance occurs, it carries with it numerous abnormalities including hypertension and dyslipidaemia (Renier, 2007).

2.1.4 Symptoms and Clinical Features of Type II DM

Diabetes mellitus usually begins gradually and progresses slowly. The early symptoms of untreated DM are related to elevated blood glucose levels and presence of glucose in the urine. High amount of glucose in the urine can lead to increase urine output hence leads to dehydration. Dehydration causes increased thirst and water intake. Inability of insulin to perform normally has effect on protein, fat and carbohydrate metabolism. Insulin as an anabolic hormone promote storage of fat and protein (Renier, 2007). A relative or absolute insulin deficiency eventually leads to weight loss irrespective of the individual having appetite or not. Diabetic patients complain of fatigue, blurred vision, nausea and vomiting. Patients are prone to developing infections of the bladder, skin and vaginal areas (Renier, 2007). Other symptoms include severe gum problems, itching, erectile dysfunction (impotence) in men, unusual sensations such as tingling or burning in the extremities.

Symptoms in children includes overweight or obesity and many children develop a skin problem called acantosis which is characterised by dark coloured patches of the skin (Harvey, 2006).

Obesity is one of the clinical features of type II DM. Recent reports have shown that obesity and DM have increased in the United State (Mokdad *et al*, 2001). In 2000, report shows that prevalence of obesity (BMI= 30 kg/m²) was 19.8 %. The prevalence of obesity with DM combined is 2.9 % (Mokdad *et al*, 2001). Individuals with central obesity are known to be predisposed to insulin resistance (Bakari *et al*, 2002). Abdominal fat is active hormonally, secreting a group of hormones called adipokines that may impair glucose tolerance. Approximately 55 % of diagnosed type II DM patients are found to be obese (Ruhe and McDonald, 2001).

2.1.5 Adverse Effects of Type II DM

Diabetes mellitus with time develops serious long term complications. These complications could be macrovascular (Chronic heart disease, cerebrovascular disease and peripheral vascular disease) and microvascular (neuropathy, nephropathy and retinopathy) (Kaneto *et al*, 1999; Rahbani – Nobar *et al*,1999; Ceriello, 2004; Aliyu *et al*, 2005; Quilliot *et al*, 2005).

A close association exist between type II DM and vascular disease (Nuttall *et al*, 1998; Gupta and Chari, 2006). The acute or chronic increase in blood glucose concentrations that result from decrease uptake of glucose into muscle and adipose tissue and an increase in hepatic output become injurious to the vasculature. Several studies have demonstrated that even mild increase in chronic (fasting) or acute (postprandial) blood glucose concentration can contribute to macrovascular injury leading to complications (Ruhe and McDonald 2001; Gupta and Chari, 2006; Benrebai *et al*, 2008). Also a close relationship has been established between poor glycaemic control and development of retinopathy and polyneuropathy (Ruhe and McDonald, 2001). Intensive glycaemic control prevents or significantly delays the development of neuropathy and diseases related to micovascular changes in type II diabetic patients (Ruhe and McDonald, 2001; Aliyu *et al*, 2005; Chertow, 2005).

Diabetic patients need to pay special attention to their `heart because DM with cardiovascular complication is the leading cause of morbidity and mortality in type II DM (Ruhe and McDonald, 2001; Lancaster *et al*, 2006). Research conducted in Australia revealed that antioxidants may also help to improve endothelial function of brachial artery in dyslipidaemic subjects with type II DM (Ruhe and McDonald, 2001).

2.1.5.1 Hyperglycaemia

The mechanism by which glucose produces its deleterious effects are not completely understood. However evidences indicates that hyperglycaemia increases OS as a result of production of free radicals beyond the protective effect of antioxidant defence system. Hyperglycaemia may promote generation of reactive oxygen species (ROS) through activation of polyol pathway and increased glucose autoxidation. This ROS concentration can cause general tissue damage (Ruhe and McDonald, 2005).

Hyperglycaemia affects non-enzymatic glycosylation of proteins. With time, in the presence of high glucose concentration, amino group of the protein reacts with glucose to form advanced glycosylation end products (AGEs). Formation of AGEs involves the participation of free radicals. AGEs can accumulate overtime and induce excess cross-linking of collagen and other extracellular proteins. Harmful effect of glycosylation occurs in a variety of tissues, modifying their structures and functions (Benrebai *et al*, 2008). Glycosylation gives rise to macrovascular and microvascular complications.

2.1.5.2 Hyperinsulinaemia

There is considerable evidence that, apart from hyperglycaemia, hyperinsulinaemia and insulin resistance can enhance free radical

generation, thus contribute to oxidative stress (Ruhe and McDonald, 2001). Studies show that oxidative stress can even have adverse effects on β -cell insulin secretion (Ruhe and McDonald, 2001; Robertson *et al*, 2003). Therefore, OS resulting from hyperglycaemia and insulin resistance can worsen type II DM by promoting further insulin resistance and decrease insulin secretion.

Hyperinsulinaemia also occurs in obese patients which may in turn induces a rise in plasma free radical production. The generation of ROS may be facilitated by hypertriglyceridaemia and hypercholestromaemia in obese individuals. Research shows that triglyceride rich-lipoproteins are more susceptible to oxidation and that pro-oxidation kinetics and a decline in antioxidant effectiveness depends on low density lipoprotein-cholesterol content (Ruhe and McDonald, 2001).

2.1.6 Complications of Diabetes Mellitus

Complications of DM has led to increased morbidity and mortality (Rahbani – Nobar *et al*,1999; Lai, 2008).

2.1.6.1 Acute complications

i) Hypoglycaemia

David (2000) stated that hypoglycaemia occurs frequently in both types I and II DM as complication of therapy. Patients on insulin therapy usually experience one to two episodes of symptomatic hypoglycaemia per week.

The two pathophysiological mechanisms that contribute to hypoglycaemia in patients with DM are (1) impairment of counter regulatory responses of glucagon and (2) deficient epinephrine secretory responses to hypoglycaemia later in the course of the disease (David, 2000). Clinical features of mild hypoglycaemia which usually occur at moderately low and easily correctable levels of blood glucose include sweating, trembling and rapid heart beat. In addition, severely low blood glucose levels can cause neurogenic symptoms such as confusion, weakness, disorientation, combativeness and in rare and worst cases coma, seizure and death.

(ii) Diabetic ketoacidosis (DKA)

In uncontrolled DM, the low insulin concentrations result in increased lipolysis and decreased re-esterification of free fatty acids thereby increasing their plasma levels (David, 2000). The increased glucagon insulin ratio enhances fatty acid oxidation in the liver. Thus increased hepatic ketone production and decreased peripheral tissue metabolism lead to acetoacetate accumulation in the blood. A small fraction undergoes spontaneous decarboxylation to form acetone, but the majority is converted to β - hydroxybutyrate (David, 2000). Accumulation of these chemicals called ketone bodies which are toxic to the body at high levels results to DKA (Harvey, 2006).

In healthy individuals, β -hydroxybutyrate and acetoacetate are present at approximately equimolar concentrations constituting virtually all the serum ketones. However, in severe DM the ratio of β -hydroxybutyrate to acetoacetate may increase to 6:1 owing to the presence of excess amount of reduced nicotinamide adenine dinucleotide (NADH) which favours β -hydroxybutyrate production (David, 2000).

Clinical features and complications of DKA may include nausea and vomiting, abnormally deep and rapid breathing with frequent sigh and rapid heart beats. If the condition persists, coma and eventually death may occur. Other serious complications of DKA include aspiration, pneumonia and respiratory distress syndrome (Harvey, 2006). Life-saving treatment uses rapid rehydration with saline solution followed by low-dose insulin and potassium replacement.

(iii) Hyperosmolar non-ketotic (HONK) coma

This is an acute complication sharing many symptoms with DKA, but an entirely different cause and different treatment. In a person with very high blood glucose levels (16 mmol/L) water is drawn out of the cells into the blood by osmosis and the kidneys dump glucose into the urine. This results in loss of water and increase in plasma osmolarity. If fluid is not replaced, the osmotic effect of high glucose levels combined with the loss of water will eventually lead to dehydration. The body's cells become progressively dehydrated as water is taken from them and excreted.

Electrolyte imbalance is also common and dangerous in this condition (Wikipedia, 2007).

2.1.6.2 Chronic Complications

(i) Microvascular complications

Microvascular complications manifest in 80 % of patients with type II DM and are the leading cause of morbidity and mortality worldwide (Remer, 2007; Benrebai *et al*, 2008). Microvascular complications include neuropathy, male impotence, nephropathy and retinopathy. Retinopathy is the leading cause of blindness, and affects 75 % of diabetics who have had the disease for more than 15 years (Kaneto *et al*, 1999; Rahbani – Nobar *et al*, 1999; Papas, 2008). Several studies have shown that DM can worsen antioxidant status hence deficiencies in some vitamins such as vitamins C and E can aggravate several complications of DM (Benrebai *et al*, 2008).

(a) Diabetic Nephropathy

This is clinically defined as persistent proteinuria in a person with diabetic nephropathy without other renal disease (Williams, 2003). This is the leading cause of renal failure in the United States. It is defined as proteinuria greater than 500 mg in 24 hrs in the presence of DM and this

is preceded by lower degrees of proteinuria or microalbuminuria. Microalbuminuria is defined as serum albumin concentration of 30 - 300 mg/24 hrs. Diabetic patients with microalbuminuria without intervention will typically progress to proteinuria and overt diabetic nephropathy. The progression occurs in both types I and II DM.

Study shows that 7 % of patients with Type II DM may already have microalbuminuria at the time they are diagnosed with DM (Gross *et al*, 2005). In the United Kingdom prospective diabetic study (UKPDS), incidence of microalbuminuria was 2 % per year in patients with type II DM and 10 years prevalence after diagnosis in 25% (Gross *et al*, 2005).

In the kidney, pathological changes involve increased glomerular basement membrane thickness, microaneurysm formation, mesangial nodule formation (Kimmelsteil-Wilson bodies) and other changes. Williams (2003) reported that diabetic nephropathy is the leading cause of end-stage renal disease requiring dialysis or transplantation. The progression to renal failure is less certain in type II than in type I diabetic patients . Diabetic nephropathy is listed as the chief cause of end stage renal disease (ESRD) in North America, Japan, Korea and most industrialized European nations. However, it has been discovered that, diabetic nephropathy accounts for 45.5 % of incidence of ESRD (Williams, 2003). Treatment with angiotensin converting enzyme (ACE)

inhibitors has been shown to decrease the risk of developing nephropathy in patients with type II DM (Gross, 2005).

(b) Diabetic neuropathy

This is a common and troublesome complication of DM leading to great morbidity and mortality and resulting in a large economic burden for care of patients with DM (Williams, 2003). This is recognized by the American Diabetes Association (ADA) as the presence of symptoms and/or signs of peripheral nerve dysfunction in people with DM after exclusion of other causes. Risk of developing diabetic neuropathy is proportional to both the magnitude and duration of hyperglycaemia (Redmon *et al*, 2009).

The nature of injury to peripheral nerves from hyperglycaemia is likely related to mechanisms such as polyol accumulation, injuries from AGEs and oxidative stress. Peripheral neuropathy in DM may manifest in several different forms including sensory focal/multifocal and autonomic neuropathies. It was observed that more than 80 % of amputations occur after ulceration or injury which can result from diabetic neuropathy (Kaneto *et al*, 1999; Rahbani – Nobar *et al*,1999).

The most common form of neuropathy in DM is chronic sensorimotor distal symmetric polyneuropathy. In this condition, patients experience burning, tingling, electrical pain and sometimes experience simple numbness with painless foot ulceration. Patients with simple numbness is

the most common form of diabetic neuropathy in developed countries (Williams, 2003).

Diabetic autonomic neuropathy also causes significant morbidity and even mortality. Neurological dysfunction can manifest by gastroparesis, constipation, diarrhoea, anhidrosis, bladder dysfunction, erectile dysfunction, exercise intolerance, resting tachycardia, silent ischaemia and even sudden cardiac arrest (Kaneto *et al*, 1999; Rahbani – Nobar *et al*,1999; Papas, 2008).

There is no specific treatment for diabetic neuropathy. The primary goal of therapy is to control symptoms and prevent worsening of neuropathy or improve glycaemic control. Kaneto *et al* (1999) and Rahbani – Nobar *et al* (1999) observed that control of hyperglycaemia and avoidance of glycaemic excursions may control symptoms of peripheral neuropathy.

(ii) Macrovascular Complications in DM

Macrovascular complications of DM include cardiovascular disease (CVD), atherosclerosis and stroke (Kaneto *et al*, 1999; Rahbani– Nobar *et al*,1999; Ceriello, 2004; Quilliot *et al*, 2005; WHO, 2005; Papas, 2008). Cardiovascular disease and stroke cause about 65 % of the death related to DM (Papas, 2008).

Atherosclerosis

The central pathological mechanism in macrovascular diseases is atherosclerosis, which leads to the narrowing of arterial walls throughout the body (Ceriello, 2004; Quilliot *et al*, 2005). This results in chronic inflammation and injury to the arterial wall in the peripheral and coronary vascular system. Monocytes produced in response to endothelial injury infiltrates arterial wall and differentiates into macrophages. This accumulates oxidized lipids to form foam cells. These foam cells stimulate macrophage proliferation and attraction of T-lymphocytes which subsequently forms lipid- rich- atherosclerotic lesion with fibrous cap. Rupture of this lesion leads to acute vascular infarction. Studies reveals that in addition to artheroma formation, there is increase platelet adhesion and hypercoagulability in type II DM. Platelet aggregation results from impaired nitric oxide generation and increased free radical formation.

Cardiovascular diseases

Diabetes mellitus increases risk of an individual to develop CVD. Research shows that CVD is the primary cause of death in people with type II DM (Kaneto *et al*, 1999; Gupta and Chari, 2006). It has been reported that atherosclerosis which is one of the most common complications is accelerated in type II DM (Rahbani – Nobar *et al*,1999; Ceriello, 2004; Gupta and Chari, 2006). Scheresten (1984) reported that

DM is associated with 2 to 3 times and 10 times higher frequency of heart disease, blindness respectively, while amputation is necessitated by DM.

Metabolic syndrome which includes abdominal obesity, hypertension, hyperlipidaemia and increased coagulability leads to type II DM and also promotes CVD (Kaneto *et al*, 1999; Balasubramanyam *et al*, 2005; Gupta and Chari, 2006). Kaneto *et al* (1999) and Gupta and Chari (2006) reported that type II diabetic women may be at higher risk of coronary heart diseases than men and that improved glycaemic control leads to decrease in macrovascular diseases.

2.1.7 Screening of Diabetes Mellitus

Patients should strive for optional glucose and blood pressure control to decrease the likelihood of developing diabetic retinopathy. Patients with DM should undergo screening for distal symmetric polyneuropathy at the time of diagnosis and yearly thereafter.

Patients with peripheral neuropathy should begin appropriate foot self care to decrease risk of ulceration. Screening for autonomic neuropathy should also be carried out. Type II diabetic patients should be screened for autonomic neuropathy. All the above are ways to achieve good glycaemic control so as to prevent long term complications of DM.

2.1.8 Diagnosis of Diabetes Mellitus

Generally, the diagnosis of DM involves good family and medical history taking, physical examination and laboratory investigations.

Multiple laboratory tests are used in the diagnosis and management of patients with DM. The qualities of the scientific evidence supporting the use of these tests varies substantially (Sacks *et al*, 2002).

2.1.8.1 *Fasting Blood Glucose Test*

The fasting blood glucose test is the preferred way to diagnose DM. It is easy to perform. The diagnosis of DM is established by the documentation of hyperglycaemia. After an individual has fasted overnight (at least 8 hours), a single sample of blood is drawn and sent to the laboratory for analysis. A glucometer can also be used to test for the level of glucose in blood. Normal fasting serum glucose level is less than 5.6 mmol/L (100 mg/dl).

In 1997, the diagnostic criteria were modified to better identify subjects at risk of retinopathy and nephropathy. The revised criteria include (a) symptoms of DM and plasma glucose ≥ 11.1 mmol/L (b) fasting plasma glucose (FPG) of ≥ 7.0 mmol/L or (c) 2 hr post load plasma glucose ≥ 11.1 mmol/L during an oral glucose tolerance test (OGTT). If any one of these three criteria is met, confirmation by repeat testing on a subsequent day is necessary to establish the diagnosis. However, the repeat testing is not necessary in patients who have unequivocal hyperglycaemia with acute metabolic decomposition.

The oral glucose tolerance test (OGTT), formerly the gold standard for diagnosing DM is now not recommended by ADA for diagnosing

either type I or II DM but continue to be recommended in a limited fashion by the World Health Organisation (WHO). It is also recommended for establishing the diagnosis of gestational DM, impaired fasting glucose (IFG) and impaired glucose tolerance (IGT). Although urine glucose is detectable in patients with grossly increased blood glucose concentrations, it provides no information about blood glucose below the variable renal glucose threshold and as such it is not recommended for routine care of patients with DM.

2.1.8.2 Oral Glucose Tolerance Test (OGTT)

This measures blood glucose level five times over a period of 2 1/2 hours. In an individual without diabetes mellitus, the glucose level rises and falls quickly. In someone with diabetes mellitus, glucose levels rise higher than normal and fails to fall fast.

People with glucose levels between normal and diabetic have impaired glucose tolerance (IGT). People with impaired glucose tolerance do not have DM, but are at high risk of progressing to diabetes mellitus. Ruhe and McDonald (2001) documented that each year, 1-5 % of people with IGT, eventually develop DM. Weight loss and exercise may help people with IGT, return their glucose levels to normal. Research shows that IGT is not simply a precursor of DM but on its own is a clinical disease entity that requires treatment and monitoring (Ruhe and

McDonald, 2001). Glucose tolerance test may lead to normal response, IGT, DM or gestational DM.

2.1.9 Diabetes Mellitus Monitoring

Regular blood glucose monitoring, urine analysis and measurement of glycated proteins and haemoglobin are important ways of controlling blood glucose and helpful means of reducing microvascular complications. Sacks *et al* (2002) observed that glycated haemoglobin (GHbA_{1c}) is more helpful than serum glucose concentration. In diabetes mellitus, studies show that reduced antioxidant levels have contributed to the development of chronic complications (Lapolla *et al*, 2008).

2.1.9.1 Urinalysis

Ketone bodies are normally present in urine and blood but in varying concentrations (example total serum ketone of <0.5 mmol/L). Increased ketone concentrations in patients with known DM or previously undiagnosed patients presenting with hyperglycaemia suggests impending or established diabetic keto acidosis (DKA), a medical emergency. Measurement of these ketones in urine and blood is recommended in patients with DM in both home and hospital settings.

Sack *et al* (2000) reported that DM is the leading cause of end-stage renal disease (ESRD) in the United States and Europe. Microalbuminuria is defined as excretion of 30-300 mg of albumin in 24 hours. Early detection of diabetic nephropathy relies upon test for urinary excretion of

albumin. Early detection of microalbuminuria also allows early intervention with a goal of delaying the onset of overt diabetic nephropathy. ADA recommends periodic qualitative (dipstick) testing for urine albumin in adults with DM. Positive tests represent “clinical albuminuria or “overt nephropathy”.

2.1.9.2 *Glycated Haemoglobin (GHbA_{1c})*

Haemoglobin A_{1c} in simple terms is sugar sticks. When it is present for a long time it is harder to get it off in the body. Sugar sticks particularly to proteins. The red blood cells that circulate in the body live for about 3 months before they are destroyed. Sugar sticks to these cells, gives idea on how much sugar is around for the preceding three months, while concentrations of other blood based glycated proteins (example glycated serum/plasma proteins known as fructosamine) also reflect mean glycaemia, but over a much shorter time than GHbA_{1c} (15-30 days). The rate of synthesis of GHbA_{1c} is principally a function of the concentration of glucose to which the erythrocytes are exposed, hence GHbA_{1c} is clinically a useful index of mean glycaemia .

In most laboratories, the normal range of GHbA_{1c} is 4 -5.9 %. In poorly controlled diabetic patients, it is 8.0 % or above and in well controlled diabetic patients it is less than 7.0 % (Awojobi *et al*, 1991). Study revealed that people with DM, who have GHbA_{1c} levels within this

range, have significantly lower incidence of complication, including retinopathy and diabetic nephropathy (Awojobi *et al*, 1991).

The benefits of measuring GHbA_{1c} is that it gives a more reasonable and stable view of what is happening over the course of time. It gives a good idea that someone is diabetic if the value is elevated. It is also used as a standard tool to determine blood glucose control in patients known to have DM. Awojobi *et al* (1991) reported that there is a direct correlation between GHbA_{1c} levels and blood glucose levels and for every 1 % reduction in GHbA_{1c} there is about 10 % decrease in relative risk of DM.

Medina *et al* (1984) reported that the risk of microvascular disease decreases by about 24 % for every 1% reduction in GHbA_{1c} values. Hence for haemoglobin, the rate of synthesis of glygated haemoglobin is a function of the concentration of glucose to which the red blood cells are exposed.

2.1.9.3 Lipid Profile

Hyperglycaemia leads to metabolic disorders characterized by alteration in metabolism of lipids. Diabetes induced disturbance in lipid profile, especially an increased subceptibility to lipid peroxidation, is responsible for increased incidence of macrovascolar complications in DM (Benrebai *et al*, 2008). Coronary artery disease (CAD) is major cause of morbidity and mortality in patients with type II DM. Consequently, measurement of serum lipids is an important recommendation for people

with DM, especially type 2, although type 1 patients are also at increased risk for cardiovascular disease. Therefore, serum lipids should be measured annually. Individuals at low risk, LDL-C < 2.6 mmol/L and HDL > 1.15 mmol/L for men and >1.4 mmol/L for women may be screened less frequently. Since many patients with DM are candidates for lipid lowering therapy, more frequent measurements may be required until normal control is achieved.

2.1.9.4 *Blood Pressure (BP)*

The measurement of blood pressure is at least as important as glycaemic control for people with type II DM in reducing the risk of diabetic complications. Good blood pressure of systolic 130 mmHg and diastolic 80 mmHg. Patients with blood pressure \geq 140/90 mmHg should be treated with drug therapy in addition to diet and life style modification.

Uncontrolled high blood pressure is a major CVD risk factor that also accelerates the progression of diabetic nephropathy (Redmon *et al*, 2009). A report from the United Kingdom prospective diabetes (UKPDS) studies showed a strong relationship between systolic blood pressure and any complication related to DM (Redmon *et al*, 2009).

2.1.10 Prognosis of Diabetes Mellitus

The diabetic patient should be educated so as to understand and participate in the control of blood glucose level, since it has been established that complications of DM are less common and severe in people who have well controlled glucose level. (Ruhe and McDonald, 2001). Factors such as smoking, elevated cholesterol levels, obesity, high blood pressure and lack of regular exercise increase the deleterious effect of DM (sacks *et al*, 2002).

Strong evidence established that there is a correlation between high glucose concentration and poor prognosis (Ruhe and McDonald 2001; Aliyu, 2005; Chertow, 2005). Study shows that the risk of death was significantly increased for patients with persistent high fasting plasma glucose (Jerry *et al*, 2002). Research finding also indicates that weight reduction is more important in the prevention of type II DM and that exercise must be accompanied with weight loss to insulin sensitivity and glucose tolerance (Ruhe and McDonald 2001).

2.1.11 Management Of Type II Diabetes Mellitus

Diabetes mellitus in all its heterogeneity has taken the centre stage as one of the ultimate medical challenges. The risk of long term complications is reduced as the level of blood glucose is carefully controlled (WHO 2000; Mokdad *et al*, 2001). Theoretically this is

achievable with combinations of diet, exercise and weight loss in type II DM. When there is high risk of CVD, life style modifications is the best in order to control blood pressure and cholesterol. This life style modification includes regular exercise cessation of smoking, consuming appropriate diet and if necessary taking any of several drugs to reduce blood pressure (Wilmer *et al*, 2003).

In type II diabetic patients, oral medication may eventually fail due to future impairment of β -cell insulin secretion. When this occurs, insulin therapy is then necessary to maintain normal glucose levels (Ruhe and McDonald, 2001; Barrett *et al*, 2002).

2.1.11.1 *Diet and Exercise*

One of the best things an individual can do for his/her health is to eat a healthy balanced diet, exercise regularly and get adequate rest. The same is true for people with normal glucose regulation and for those with pre-diabetes, as it is well known that eating right, exercising and losing weight can help control blood glucose levels and prevent onset of DM (Mokdad *et al*, 2001; Deets, 2004). Physical exercise is also recommended to realize optimal glucose control benefits in type II diabetic patients (WHO, 2000; Deets, 2004).

Many people can control their blood glucose through proper food selection and exercise. Because obesity and physical activity are the major risk factors of development of type II DM, the most effective method of

prevention, and management is through weight loss and exercise. Studies in Caucasians show that 65-75 % cases of DM could be avoided if ideal weight is not exceeded and even moderate weight loss is associated with significantly reduced risk of DM (Ruhe and McDonald, 2001).

2.1.11.2 Pharmaceutical Intervention

Most cases of type II DM could be prevented or treated effectively if individuals at risk adhere to a strict diet and exercise regiment but in the real world this does not happen. Genetic, environmental and social factors combine to make dieting difficult and exercise inconvenient. There are some individuals that their diet and exercise alone cannot manage their hyperglycaemia. Faced with these realities pharmaceutical industry decided to develop oral hypoglycaemic drugs agents which act to lower blood glucose.

Generally, improved glycaemic control is accomplished pharmacologically by stimulating the release of insulin from functioning β -cells in pancreatic islet and improving insulin sensitivity in peripheral tissues. The drugs include sulfonylurea, melginitinides, biguanides and thiazolidinediones. The drugs act by reducing hepatic glucose output and decrease insulin resistance. This has been used with varying degree of success (Ruhe and McDonald, 2001).

2.1.11.3 Antioxidant Therapeutic Approaches

In healthy individuals, the body has defense mechanisms that control plasma ROS concentrations under most conditions. However, in individual with type II DM, increased ROS generation and marked reduction in antioxidant defenses result in OS, which lead to many of the deleterious effects of Diabetes Mellitus. Hence, any treatment and management should include the direct or indirect reduction of OS. Some of the drugs like troglitazone and sulfonylurea posse antioxidant activity (Ruhe and McDonald, 2001). Antioxidant nutrients may complement the above therapies. Studies show that consumption of fruit and vegetables increases TAS (Ruhe and McDonald, 2001; Aliyu *et al*, 2005). Increased TAS in the system also reduces insulin resistance (Ruhe and McDonald, 2001). Studies show that vitamin E prevents or at least delay many of the vascular complications associated with type II DM (Ruhe and McDonald, 2001).

2.2 ANTIOXIDANTS

Medical nutrition therapy (MNT) guidelines acknowledge the need to identify deficiencies of antioxidant vitamins. However, the guidelines observe that such identification is difficult. Thus there is evidence that available clinical laboratory tests for antioxidant vitamins C and E are not been carried out in clinical practice (Nwose, 2009).

Vitamins C and E tests can be used to ascertain the levels of antioxidant vitamins (Nwose, 2009). Since DM is associated with increased

lipid peroxidation, this has been implicated in the pathogenesis of DM complications. To control these, several antioxidant protective mechanism like vitamins C and E exist (Ahmad *et al*, 2003).

2.2.1 Antioxidants And Diabetes Mellitus

Antioxidants are free radical scavengers, while DM is a free radical associated disease. Antioxidants have the power to help prevent DM as well as help manage it. Studies show that a number of antioxidants participate in the protection of human body against free radical pathology and its consequences (Aliyu *et al*, 2005).

Investigation in DM patients revealed oxidative stress (OS) load (Aliyu *et al*, 2005). It is believed that DM is associated with increased OS as increased blood concentration of thiobarbituric acid reactive substances (TBARS) have been reported (Lai, 2008). Disturbances of antioxidant enzymes and low antioxidant vitamin levels have been reported in DM (Sief and Youseef, 2004). Decreased lipid peroxidation and improved antioxidant status may be one mechanism by which treatment of DM contributes to the prevention of diabetic complications (Amstrong *et al*, 1996).

Reactive oxygen species (ROS) are known to attack cell membranes, resulting in the propagation of lipid peroxidations. Studies revealed oxidative destruction of subcellular membrane lipids along with other types of intracellular oxidative damage in normal aging process and in

pathophysiology of many chronic illnesses like DM (Nuttall *et al*, 1998; Aliyu *et al*, 2005). Complex antioxidant mechanisms, including antioxidant vitamins exist to limit the effects of these reactions. Oxidative damage due to free radicals is associated with vascular disease in people with type II diabetes mellitus (Nuttall *et al*, 1998; Chertow, 2004; Lai 2008).

Oxidative stress and free radicals result from either an increase production or decrease in clearance. An excess of free radicals is detrimental to cell function including β -cells, endothelial cells, fat, muscle and nerve cells (Chertow, 2004).

Decrease production or increase clearance should reduce the net amount of free radicals and cell damage. Several potential sources of free radicals production in diabetes mellitus include autoxidation of plasma glucose, leucocytes activation and increased transition metal bioavailability (Lai, 2008).

2.2.2 Total Antioxidant Status (TAS)

Total antioxidant status includes antioxidant defense system (enzymes, vitamins and minerals). It is believed that DM is associated with increased OS and increased blood concentrations of thiobarbituric acid reactive substances (TBARS) which is a measure of lipid peroxidation (Ifeoma *et al*, 2007; Lai, 2008). Studies revealed that TAS in type II DM is low and this could be attributed to low levels of vitamins C and E or other

micro-nutrients in blood (Lai, 2008). In Japan, a study revealed that antioxidant treatment is useful and can exert beneficial effects in DM with a preservation of *in vivo* beta-cell function (Kaneto 1999). Similarly, in Brazil, a study revealed lower plasma antioxidant activity in DM than in healthy individuals (Medina *et al*, 2007). In Nigeria, study shows that hypertension is associated with increased oxidative stress and depleted non-enzymatic antioxidant status (Nwanjo *et al*, 2007).

In Sokoto, northern Nigeria, study shows that vitamins A, C and E are significantly lower in type II diabetic patients than controls. It also revealed high prevalence of vitamins C and E deficiency among type II diabetic subjects (Aliyu *et al*, 2005). The study also revealed that type II diabetic subjects have low serum levels of antioxidant vitamins and the concentration of these vitamins correlated negatively with blood glucose levels (Aliyu *et al*, 2005). Akinsoun and Bolajoko (2007), reported decreased total antioxidant status (TAS) in type II diabetic Nigerians and proposed that reduction in free radical activity may probably minimize the chronic complications in diabetic patients.

2.2.3 Vitamin C

Ascorbic acid is of great importance in biochemical reactions as a reducing agent. For example, recycling of antioxidants such as vitamin E by ascorbic acid has been shown to be protective against oxidative stress (Moeslinger *et al*,1995).

Glucose and vitamin C have similar structure and so vitamin C can replace glucose in many chemical reactions hence prevent non-enzymatic glycosylation of proteins (Afkharni–Ardekan and Shojaoddiny-Ardekani, 2007). In addition Vitamin C has been studied in pigs and found out to act as regulator of catabolism of cholesterol and bile acid, hence vitamin C is an important factor in lipid regulation (Afkharni-Ardekan and Shojaodding-Ardekani, 2007).

Several studies revealed that vitamin C is decreased in DM patients (Afkhami-Ardekan and Shojoddiny-Ardekami, 2007). In India, the levels of vitamin C is significantly reduced in diabetic patients with complications than those without complications (Gupta and Chari, 2006). Study also shows that vitamin C as an antioxidant may help in importing plasma glucose by reducing insulin resistance hence lowering oxidative stress in type II diabetic patients (Ifeoma *et al*, 2007). Vitamin C is found to protect, prevent and reduce the extent of oxidative destruction of cellular tissue (Ifeoma *et al*, 2007). Futhermore, study shows that vitamin C was found to prevent the onset of type II DM (Ifeoma *et al*, 2007).

2.2.4 Vitamin E

Vitamin E is an antioxidant vitamin and it is also known as α -tocopherol. However, vitamin E has been shown to play a role in preventing DM (Deets, 2004; Ifeoma *et al*, 2007). Vitamin E protects,

prevents or reduces the extend of oxidative destruction of cellular tissues (Ifeoma *et al*, 2007). Vitamin E is considered as the most important lipid-soluble exogenous antioxidant in humans. It is regenerated by Vitamin C in the system (Lai, 2008).

If the level of vitamin E is reduced in the system, it leads to increase free radical production which later mediates tissue injury in DM (Ifeoma *et al*, 2007). Adults with metabolic syndrome have been shown to have suboptimal concentrations of vitamin E (Ifeoma *et al*, 2007). Diabetes mellitus is associated with production of high levels of lipid peroxidation products like malondialdehyde (MDA) and reduced glutathione (GSH). Vitamin E increases GSH and reduces MDA hence reduce microvascular and macrovascular complications (Ifeoma *et al*, 2007).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 SUBJECTS

A total of 281 subjects were recruited for the study. These consist of 181 known diabetic patients attending Medical Out-Patient Department (MOPD) clinic of ABUTH, Shika, Zaria and 100 apparently healthy individuals. The apparently healthy individuals (controls) were recruited from the population of staff and students of ABU and ABUTH, Zaria.

3.1.1 Inclusion Criteria

Individuals with established type II DM and had well controlled hypertension who agreed to participate were included in the study. Apparently healthy non diabetic age-matched subjects were recruited as controls.

3.1.2 Exclusion Criteria

Individuals with gestational and type I diabetes mellitus were excluded from the study. All those who declined to give consent for inclusion were also excluded from the study.

3.1.3 Informed Consent

Informed written consent was obtained from all subjects before inclusion using approved protocol (Appendices I and II).

3.2 SAMPLE SIZE DETERMINATION

The sample size for the study was determined from a standard formula for the calculation of minimum sample size (Oyejide, 1992).

$$n = \frac{(Z_{1-\alpha})^2 (p) (1-p)}{d^2}$$

Where: n= sample size, $(Z_{1-\alpha})$ = the value standard normal deviation which at 95 % confidence level has been found to be 1.96, d = degree of confidence 0.02,

p = percentage prevalence of DM in Zaria = 1.6 % (Bakari *et al*,1999).

Therefore the sample size (n) is calculated as:

$$n = \frac{(1.96)^2 (0.016) (1-0.016)}{(0.02)^2}$$

$$n = 162$$

Therefore, the calculated sample size for the study is 162. However 181 was used as sample size for better analysis.

3.3 ETHICAL APPROVAL

The approval of the study was obtained from the Ethical Committee of the Faculty of Medicine, Ahmadu Bello University, Zaria in accordance with Helsinki declaration (Appendix III).

3.4 SAMPLING TECHNIQUES

Arrangement was made with the clinicians whereby subjects who satisfy the study inclusion criteria were selected. Structured

questionnaires were administered to the study population. Information including the name, age, gender, ethnic group, height, weight, blood pressure, duration of diabetes and complications were obtained through personal interviews and this was followed by specimen collection. Glycaemic control was assessed by glucose and glycated haemoglobin measurements. The findings were all documented (Appendix III).

3.5 SPECIMEN COLLECTION AND PROCESSING

Blood specimen (about 10ml) for the biochemical measurements was collected from peripheral vein (antecubital venepuncture). In this case, the antecubital fossa was cleaned with methylated spirit and allowed to dry. A tourniquet was then applied a few centimeters above the antecubital fossa to distend the veins. Blood was taken using a sterilized 10 ml syringe and 22-G needle. About 3 ml of whole blood was dispensed into EDTA bottle for the estimation of glycated haemoglobin (GHbA_{1c}). The coagulated whole blood was then spun using a centrifuge, the serum was removed, transferred to clean labeled Bijou bottles and analysed as soon as possible. Specimens that were not assayed within 24 hours of collection due to logistic problems were stored frozen at -20 °C until needed for analysis.

3.6 EQUIPMENT

Hettich Universal 32 Centrifuge (Germany) was used to spin blood samples. Beckman Coulter DU-520 general purpose UV/VIS Spectrophotometer (Germany) was used for the measurements of serum glucose, glycated haemoglobin while Bio-rad PR 5100 microplate reader was used for measurement of TAS. Shimadzu UV-Visible spectrophotometer 2550 was used for vitamins C and E measurements.

3.7 CHEMICALS/REAGENTS

The chemicals used for the determinations of fasting serum glucose, glycated haemoglobin, and total antioxidant status were procured from Randox Company Limited. Vitamins C and E reagents were procured from BDH Chemicals Limited (Poole Dorset England). All the chemicals and reagents were of analytical grade or higher.

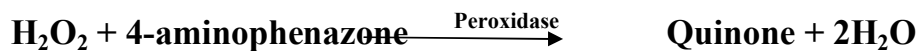
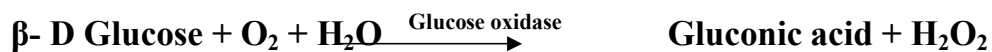
3.8 ANALYTICAL METHODS

3.8.1 *Measurement of Serum Glucose*

Serum glucose was measured using enzymatic method of Trinder (1969).

3.8.1.1 Principle

Glucose oxidase catalyses the oxidation of glucose to give hydrogen peroxide (H_2O_2) and gluconic acid. In the presence of peroxidase, H_2O_2 is broken down and the oxygen released reacts with 4- aminophenazone (4- aminoantipyrine) and phenol to produce a pink coloured quinoneimine complex that can be measured spectrophotometrically at 505 nm.



3.8.1.2 Procedure

Into each of clean test tubes labeled test, standard and blank, 0.5 ml glucose solution was placed. Then 0.05 ml of serum sample, standard solution and distilled water was added respectively. These were incubated at 37 °C for 10 minutes and the absorbance read at 505 nm. The results were calculated as follows:

$$\text{Serum glucose (mmol/l)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Concentration of Standard}$$

3.8.2 Measurement of Serum Glycated Haemoglobin (GHbA_{1c})

GHbA_{1c} was measured using micro column method (Trivelli *et al*, 1971).

3.8.2.1 Principle

After preparing the haemolysate where the labile fraction is eliminated, GHbA_{1c} is specifically eluted after washing away the HbA_{1a} + b fraction and is quantified by direct photometric reading at 450 nm.

3.8.2.2 Procedure

a. Haemolysate preparation and labile fraction elimination

The column and reagent were brought to room temperature. Fifty (50) μ l of blood and 1.2 ml of reagent were pipetted into a test tube. The contents were then thoroughly mixed and incubated for 15 minutes at room temperature.

b. Column preparation

The upper cup of the column was removed and the tip of the button snapped. The upper disc was pushed down carefully just to touch the resin surface. Precaution was taken not to compress the column by using the flat end of a pipette. The column was allowed to drain completely to waste.

c. GHbA_{1c} separation

Fifty (50) μ l of haemolysate and 200 μ l of Reagent 2 were respectively pipetted carefully on the upper filter. The column was placed over a tube and 4 ml of Reagent 3 was added and then GHbA_{1c} fraction was eluded and collected. The collected content was thoroughly mixed and

the absorbance (A) of the GHbA_{1c} measured at 415 nm after zeroing the machine with distilled water.

d. Total haemoglobin (HbA_{1c})

Into a test tube, 12 ml of Reagent 3 and 50 µl of haemolysate were respectively transferred and then thoroughly mixed. The absorbance of the resulting GHbA_{1c total} was read at 415 nm against distilled water.

The results were calculated using the following expression:

$$\% \text{ GHbA}_{1c} = A_{\text{Hb1c}} \times V_{\text{Hb1c}} / A_{\text{Hb-total}} \times V_{\text{Hb-total}} \times 100$$

$$\text{Or } \% \text{ GHbA}_{1c} = A_{\text{Hb1c}} / A_{\text{Hb-total}} \times 100/3,$$

Where :

$V_{\text{HbA}_{1c}}$ = Volume of GHbA_{1c} =(4 ml), $V_{\text{Hb-TOTAL}}$ = Volume of Hb_{TOTAL}= (12 ml), $A_{\text{HbA}_{1c}}$ = Absorbance of GHbA_{1c} and $A_{\text{Hb-TOTAL}}$ = Absorbance of GHbA_{1c}

3.8.3 Measurement of Serum Total Antioxidant Status (TAS)

Serum TAS was measured using rapid colourimetric 3-[4,5-dimethylthiazol-2-yl]-2,5-dimethyl tetrazolium bromide (MTT) assay by Mosmann (1983).

3.8.3.1 Principle

When MTT dye in phosphate buffered saline (PBS) is incubated with serum for 30 minutes at 37 °C, the dye is reduced to a blue coloured

product. The reaction is stopped by the addition of hydrochloric acid in isopropanol and the absorbance read at 580 nm.

3.8.3.2 Procedure

Fifty (50) μl of Serum, standard and PBS were each placed into micro wells respectively and 50 μl of dye solution (5 mg/ml in PBS) was added to each. The mixtures were then incubated for 30 minutes at 37 °C. The reaction was terminated by the addition of 1.0 ml of hydrochloric acid (0.04 N) in isopropanol to each micro well. The mixtures were allowed to stand for 10 minutes at room temperature. The absorbance were measured at 580 nm after zeroing the machine with blank.

Calculation

$$\text{Concentration of TAS} = \frac{\Delta \text{Abs}_{(\text{Std 1})} - \Delta \text{Abs}_{(\text{Sample})}}{\Delta \text{Abs}_{(\text{Std 1})} - \Delta \text{Abs}_{(\text{Std 2})}} \times \text{STD 2 value.}$$

Where:

Δ Absorbance Standard 1= (2nd absorbance of std1 - 1st Absorbance of Std 1)

Δ Absorbance Standard 2= (2nd absorbance of std2 - 1st Absorbance of Std 2)

Δ Absorbance Standard1= (2nd absorbance of Sample - 1st Absorbance of sample1)

3.8.4 *Measurement of Serum Vitamin C*

Serum vitamin C was determined by spectrophotometric method (Moeslinger *et al*, 1995).

3.8.4.1 Principle

Ascorbic acid absorbs maximally at 478.5 nm in methanol.

3.8.4.2 Procedure

To 1 ml of serum, 5 ml of methanol was used to extract vitamin C at 37°C for 15 minutes using a hot plate. This was allowed to stand and cool for 5 minutes before reading the concentration of the individual samples at 478.5 nm against a standard curve which was prepared with standard solution of ascorbic acid.

3.8.5 *Measurement of Serum Vitamin E*

Serum vitamin E (tocopherol) was measured by spectrophotometric method (Dahot *et al*, 1990).

3.8.5.1 Principle

α -tocopherol absorbs maximally at 285 nm in methanol.

3.8.5.2 Procedure

To 1 ml of serum, 5 ml of methanol was used to extract vitamin E at 37°C for 15 minutes using hot plate. This was allowed to stand and cool for 5 minutes before reading the concentration of the individual samples at 285 nm against a standard curve which was prepared with standard α -tocopherol. Absorbance was read in a Shimadzu UV-Visible spectrophotometer 2550.

3.9 QUALITY CONTROL (QC)

All analytical tests were done according to the standard operating procedure. It involves the use of inter and intra-run of control sera along with the samples. Pre-analytical and post analytical precautions were observed.

3.10 REFERENCE VALUES

The reference values were obtained by calculation, mean \pm 2SD after probit plot (Fig.3 to 5).

3.11 STATISTICAL ANALYSIS

The data obtained were treated accordingly using Statistical Program for the Social Sciences (SPSS 13.0) for windows (SPSS Inc., Chicago, 16). Serum total antioxidant status, vitamins C and E, glucose and glycated haemoglobin concentrations obtained from diabetic patients were compared with those of apparently healthy individuals (controls) using the two tailed student's t- test. Similarly, the sexes and presence/absence of complications in patients and controls were analysed

using the two tailed student's t- test. One way analysis of variance (ANOVA) was employed to compare the results of different age groups of diabetic patients as well as controls. Correlations between TAS and glucose as well as glycated haemoglobin were carried out using Pearson's linear correlation analysis. A p-value of equal to or less than 0.05 ($p \leq 0.05$) was considered statistically significant.

CHAPTER FOUR

4.0 RESULTS

4.1 ANALYSIS OF THE STUDY POPULATION

One hundred and eighty one diabetic patients aged 20-80 years were recruited for the study. These were made up of 52 males (29 %) with mean age 53 years and 129 females (71%) with mean age 53 years (Table 4.1).

4.2 CLINICAL PARAMETERS IN DIABETIC PATIENTS AND CONTROLS

The mean values of clinical parameters in diabetic patients and controls are shown in Table 4.2. The mean values of BMI and DBP in diabetic patients were significantly higher ($p < 0.05$) than corresponding values in the control subjects. However, the value of SBP in diabetic patients was similar to that of controls ($p > 0.05$).

4.3 CLINICAL PARAMETERS IN DIABETIC PATIENTS WITH GOOD AND POOR GLYCAEMIC CONTROL

The mean value of DODM in diabetic patients with poor control was significantly higher ($p < 0.05$) than that of good control as shown in table 4.3. The mean values of other parameters in both good and poor glucose controls were similar ($p > 0.05$).

4.4 CLINICAL PARAMETERS IN DIABETIC PATIENTS WITH GOOD AND

POOR GHbA_{1c} CONTROL

Table 4.4 shows mean values of age, body mass index, duration of diabetes, systolic and diastolic blood pressure of diabetic patients with good and poor glycosylated haemoglobin control. All the clinical parameters in both groups were similar ($p > 0.05$).

Table 4.1: Gender distribution of diabetic patients

SUBJECTS (years)	n	PERCENTAGE (%)	MEAN AGE
diabetics			
Male	52	29	
53± 1.4			
female	129	71	53± 1.0

n=Number of patients.

Table 4.2: Clinical parameters (Mean±SEM) in diabetic patients and controls

SUBJECTS	N	Age (years)	BMI (Kg/m²)	DODM (years)	SBP (mmHg)	DBP (mmHg)
Patients	181	53 ± 0.8	28.4 ±0.4*	5 ± 0.4	118 ±0.6	78 ±0.5*
Controls	100	52 ± 0.8	22.8 ±0.3*	—	117 ±0.8	74 ± 0.8*
p-value		<0.05	<0.05	—	>0.05	<0.05

n=Number of subjects, BMI=body mass index, DODM =duration of diabetes mellitus, SBP= systolic blood pressure, DBP= diastolic blood pressure, SEM=standard error of mean and NA=not applicable.

*** statistically significant (p< 0.05).**

Table 4.3: Clinical parameters (Mean±SEM) in diabetic patients with good and poor glucose control

PATIENT S	N	AGE (years)	BMI (Kg/m²)	DODM (Years)	SBP (mmHg)	DBP (mmHg)
Good control (≤ 6.0)	92	54± 1.0	28.7±0.5	4± 0.4*	118 ±0.9	77±0.7
Poor Control (> 6.0)	89	52± 1.2	28.1±0.5	6±0.6*	118± 0.9	78±0.8
p-value		> 0.05	>0.05	<0.05	>0.05	>0.05

n=Number of subjects, BMI=body mass index, DODM=duration of diabetes mellitus, SBP- systolic blood pressure, DBP- diastolic blood pressure and SEM=standard error of mean. Good control = values below mean -3SD while poor control = values above mean -3SD

*** statistically significant (p< 0.05).**

Table 4.4: Clinical parameters (Mean±SEM) in diabetic patients with good and poor glycated haemoglobin control

PATIEN TS	N	AGE (years)	BMI (Kg/m²)	DODM (Years)	SBP (mmHg)	DBP (mmHg)
Good control (≤ 7.0)	59	54± 1.3	28.8± 0.6	4± 0.6*	118±1.1	77±1.0
Poor Control (> 7.0)	122	53± 1.0	28.2±0.5	6± 0.4*	119± 0.8	79± 0.6
p-value		>0.05	>0.05	<0.05	>0.05	>0.05

n=Number of subjects, BMI =body mass index, DODM =duration of diabetes mellitus, SBP= systolic blood pressure, DBP= diastolic blood pressure and SEM=standard error of mean. Good control = values below mean -3SD while poor control = values above mean -3SD.

*** statistically significant (p< 0.05).**

4.5 CLINICAL PARAMETERS IN DIABETIC PATIENTS ACCORDING TO THEIR GENDER

Table 4.5 shows mean values of clinical parameters in diabetic patients according to gender. All the parameters in male and female subjects were similar ($p > 0.05$).

4.6 CLINICAL PARAMETERS IN CONTROL SUBJECTS ACCORDING TO THEIR GENDER.

Table 4.6 shows mean values of clinical parameters in control subjects according to gender. All the parameters in male and female subjects were similar ($p > 0.05$).

4.7 CLINICAL PARAMETERS IN DIABETIC PATIENTS WITH AND WITHOUT COMPLICATIONS

The clinical parameters in diabetic patients with complications were found to be similar ($p > 0.05$) with that of those without complications as presented in table 4.7. However, the mean age and duration of diabetes of those with complications were significantly higher ($p < 0.05$) than in those without complications.

4.8 SERUM BIOCHEMICAL ANALYTES IN DIABETIC PATIENTS AND CONTROLS

The mean values of TAS, GHbA_{1c}, FBG, vitamins E and C in diabetic

patients and controls are shown in Table 4.8. The mean values of TAS, Vitamins E and C were significantly higher ($p < 0.05$) in controls than in diabetic subjects. However, the mean values of FBG and GHbA_{1c} were significantly higher ($p < 0.05$) in diabetic patients than control subjects

4.9 BIOCHEMICAL ANALYTES IN DIABETIC PATIENTS WITH GOOD AND POOR GLUCOSE CONTROL

The mean values of TAS, vitamins E and C in diabetic patients with good and poor glycaemic control are shown in Table 4.9. Vitamin E in patients with good control was found to be significantly higher ($p < 0.05$) than in patients with poor control. Other biochemical analytes were similar ($p > 0.05$).

Table 4.5: Clinical parameters (Mean±SEM) in diabetic patients according to gender

PATIENTS	N	AGE (years)	BMI (Kg/m ²)	DODM (Years)	SBP (mmHg)	DBP (mmHg)
Male	52	53± 1.4	27.6±5.7	6± 0.8	119±1.2	78±1.1
Female	129	53± 1.0	28.7±0.5	5± 0.4	117± 0.8	77± 0.6
p-value		>0.05	>0.05	>0.05	>0.05	>0.05

n=Number of subjects, BMI=body mass index, DODM=duration of diabetes mellitus, SBP=systolic blood pressure, DBP=diastolic blood pressure, SEM=standard error of mean and NA=not applicable.

Table 4.6: Clinical parameters (Mean±SEM) in control subjects according to gender

CONTRO LS	N	AGE (years)	BMI (Kg/m²)	SBP (mmHg)	DBP (mmHg)
Male	58	50 ± 1.0	22.8±0.4	118±1.1	75±1.1
Female	42	52 ± 1.3	22.8±0.5	117± 1.2	77± 1.2
p-value		>0.05	>0.05	>0.05	>0.05

n=Number of subjects, BMI=body mass index, DODM=duration of diabetes mellitus, SBP= systolic blood pressure, DBP= diastolic blood pressure, SEM=standard error of mean and NA=not applicable.

Table 4.5
poor gluc

Table 4.7: Clinical parameters (Mean±SEM) in diabetic patients with and without complications

PATIENTS	n	AGE (years)	BMI (Kg/m²)	DODM (Years)	SBP (mmHg)	DBP (mmHg)
With complications	25	58± 2.3*	27.7±0.8	13± 1.4*	117±1.9	77±1.4
Without complications	156	53± 0.8*	28.5±0.4	4± 0.2*	118 ± 0.7	77± 0.6
p-value		<0.05	>0.05	<0.05	>0.05	>0.05

n=Number of subjects, BMI=body mass index, DODM=duration of diabetes mellitus, SBP=systolic blood pressure, DBP=diastolic blood pressure, SEM=standard error of mean and NA=not applicable.

*** statistically significant (p< 0.05).**

Table 4.8: Biochemical analytes (Mean±SEM) in diabetic patients and controls

SUBJECTS	N	TAS (mmol/L)	FBG (mmol/L)	GHbA_{1c} (%)	Vit. C (Mg/ml)	Vit E (g/l)
Patients	181	33.3± 0.5*	7.0±0.3 *	8.2± 0.2*	3.1±0.1 *	53.2±2.2*
Controls	100	39.4± 0.8*	4.1±0.8 *	5.0±0.1*	3.6±0.2 *	61.5±2.9*
p-value		<0.05	<0.05	<0.05	<0.05	<0.05

n=Number of subjects, TAS = total antioxidant status, FBG=fasting blood glucose,

GHbA_{1c} = glycated haemoglobin, Vit E= vitamin E, Vit C = vitamin C
SEM=

standard error of mean.

* statistically significant (p< 0.05).

Table 4.9 Biochemical analytes (Mean±SEM) in diabetic patients with good and poor glucose control

PATIEN TS	N	TAS (mmol/L)	Vit. C (Mg/ml)	Vit. E (g/L)
Good control (≤ 6.0)	92	33.3± 0.7	3.3±0.1	44.6±3.0*
Poor Control (> 6.0)	89	32.2± 0.7	3.2± 0.2	41.8±2.7*
p-value		>0.05	>0.05	<0.05

n=Number of subjects, TAS = total antioxidant status, FBG=fasting blood glucose, GHbA_{1c} = glycated haemoglobin, Vit E= vitamin E, Vit C = vitamin C and SEM=standard error of mean. Good control = values below mean -3SD while poor control = values above mean -3SD.

* statistically significant (p< 0.05).

4.10 BIOCHEMICAL ANALYTES IN DIABETIC PATIENTS WITH GOOD AND POOR GHbA_{1c} CONTROL

The mean values of TAS, vitamins E and C in diabetic patients with good and poor glycaemic control are shown in Table 4.10. Vitamin E in patients with good control was found to be significantly higher ($p < 0.05$) than in patients with poor control. Other biochemical analytes were similar ($p > 0.05$).

4.11 BIOCHEMICAL ANALYTES (MEAN \pm SEM) IN DIABETIC PATIENTS ACCORDING TO GENDER

The mean values of biochemical analytes in diabetic patients as shown in table 4.11. The male and female subjects have similar values except for serum FBG that was significantly higher ($p < 0.05$) in female.

4.12 BIOCHEMICAL ANALYTES IN CONTROL SUBJECTS ACCORDING TO GENDER

The mean values of biochemical analytes in control subjects reveal that all the analytes in both male and female subjects are similar. However, vitamin C in was found to be significantly higher ($p < 0.05$) in females (table 4.12).

4.13 BIOCHEMICAL ANALYTES IN DIABETIC PATIENTS WITH AND WITHOUT COMPLICATIONS

The mean values of all the biochemical analytes in diabetic patients with complications and those without complications are similar (table

4.13). Except vitamin E which was significantly higher ($p < 0.05$) in patients without complications than those with complications.

Table 4.10: Biochemical analytes (Mean \pm SEM) in diabetic patients with good and poor GHbA_{1c} control

PATIENTS	N	TAS (mmol/L)	Vit. C (Mg/ml)	Vit. E (g/L)
Good control (≤ 7.0)	93	33.4\pm 0.6	3.1\pm0.1	44.5\pm2.7*
Poor Control (> 7.0)	88	33.1\pm 0.6	3.0\pm 0.1	41.7\pm2.8*
p-value		>0.05	>0.05	<0.05

n=Number of subjects, TAS = total antioxidant status, GHbA_{1c} = glycated haemoglobin, Vit E= vitamin E, Vit C = vitamin C and SEM=standard error of mean. Good control = values below mean -3SD while poor control = values above mean -3SD

*** statistically significant ($p < 0.05$).**

Table 4.11 Biochemical analytes (Mean±SEM) in diabetic patients according to gender

PATIEN TS	N	TAS (mmol/L)	FBG (mmol/L)	GHbA_{1c} (%)	Vit. C (Mg/ml)	Vit. E (g/L)
Male	52	33.9± 1.0	5.9±0.4*	7.9± 0.3	3.1±0.2	41.3±2.7
Female	129	33.0± 0.6	7.4±0.4*	8.4±0.3	3.1± 0.1	43.9±2.6
p-value		>0.05	<0.05	>0.05	>0.05	>0.05

n=Number of subjects, TAS = total antioxidant status, FBG=fasting blood glucose, GHbA_{1c} = glycated haemoglobin, Vit E= vitamin E, Vit C = vitamin C and SEM=standard error of mean.

*** statistically significant (p< 0.05).**

Table 4.12 Biochemical analytes (Mean±SEM) in control subjects according to gender

CONTROL S	N	TAS (mmol/L)	FBG (mmol/L)	GHbA_{1c} (%)	Vit. C (Mg/ml)	Vit. E (g/L)
Male	58	39.6±1.0	4.1±0.9	3.17±0.2	3.1±0.2*	65.6±4.2*
Female	42	39.0± 1.2	4.0±0.1	4.18±0.3	4.2±0.3*	55.9±3.6*
p-value		>0.05	>0.05	>0.05	<0.05	<0.05

n=Number of subjects, TAS = total antioxidant status, FBG=fasting blood glucose,
 GHbA_{1c} = glycated haemoglobin, Vit E= vitamin E, Vit C = vitamin C and SEM=standard error of mean.

* statistically significant (p< 0.05).

Table 4.13 Mean values for biochemical parameters (Mean±SEM) in diabetic patients with and without complications

PATIENTS	n	TAS (mmol/L)	FBG (mmol/ L)	GHbA_{1c} (%)	Vit. C (Mg/ml)	Vit. E (g/L)
With complication	25	32.8± 0.6	7.7±0.7	8.3± 0.5	2.91±0.3	42.5±2.1*
Without complication	15 6	34.4± 1.1	6.8±0.3	8.22±0.2	3.1±0.1	49.5± 7.0*
p-value		>0.05	>0.05	>0.05	<0.05	>0.05

n=Number of subjects, TAS = total antioxidant status, FBG=fasting blood glucose,

GHbA_{1c}= glycated haemoglobin, Vit E= vitamin E, Vit C = vitamin C and SEM=standard error of mean.

* statistically significant (p< 0.05).

4.14 CALIBRATION CURVE FOR VITAMIN C ESTIMATION

A plot of absorbance versus concentration of vitamin C standard measured in duplicate is shown in fig 4.1 on a Shimadzu UV-visible spectrophotometer 2550.

4.15 CALIBRATION CURVE FOR VITAMIN E ESTIMATION

A plot of absorbance versus concentration of vitamin E standard measured in duplicate is shown in fig 4.2 on a Shimadzu UV-visible spectrophotometer 2550.

4.16 THE REFERENCE VALUES FOR TAS, VITAMINS C AND E

The reference values for TAS, vitamins C and E were 15.7 – 63.1 mmol/L, 0.2 – 7.0 mg/ml, 3.7- 119.3 g/L respectively as shown in table 4.14. The probit plots for TAS, vitamins C and E are shown in figures 3 to 5 respectively.

4.17 CORRELATION ANALYSIS IN DIABETIC PATIENTS (PEARSON'S CORRELATION)

The correlations between TAS and age was statistically significant ($r=0.173$, $p < 0.05$), as well as DODM ($r= 0.185$, $p < 0.05$) in diabetic patients (figures 6 to 7).

Table 4.14 Reference values of Serum antioxidants from the present study.

Biochemical analytes	Reference values	Units
TAS	15.7 – 63.1	mmol/L
Vitamin C	0.2 – 7.0	mg/ml
Vitamin E	3.7 – 119.3	g/L

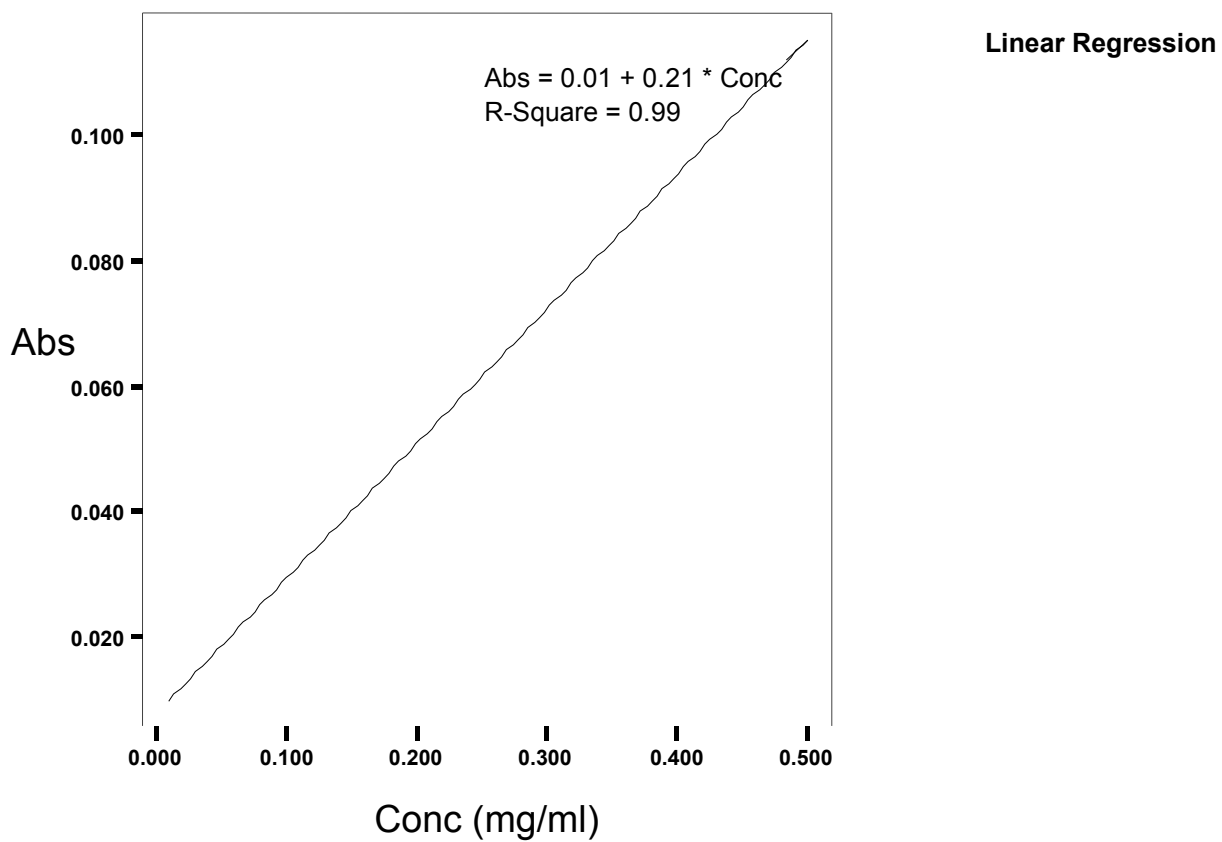


Fig 4.1: Standard curve for vitamin C estimation on shimadzu uv-visible spectrophotometer 2550

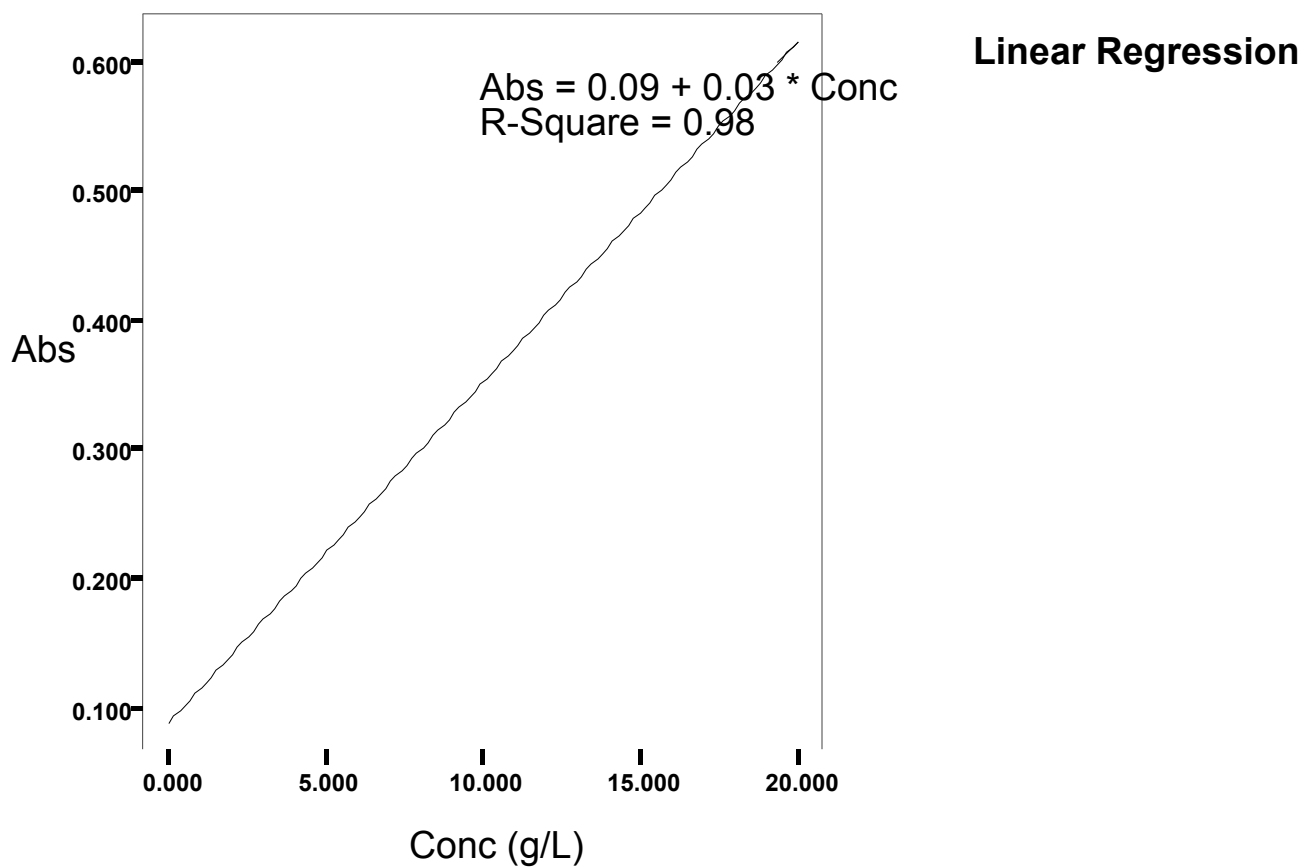


Fig 4.2: Standard curve for vitamin E estimation on shimadzu uv-visible spectrophotometer 2550

Normal P-P Plot of TAS

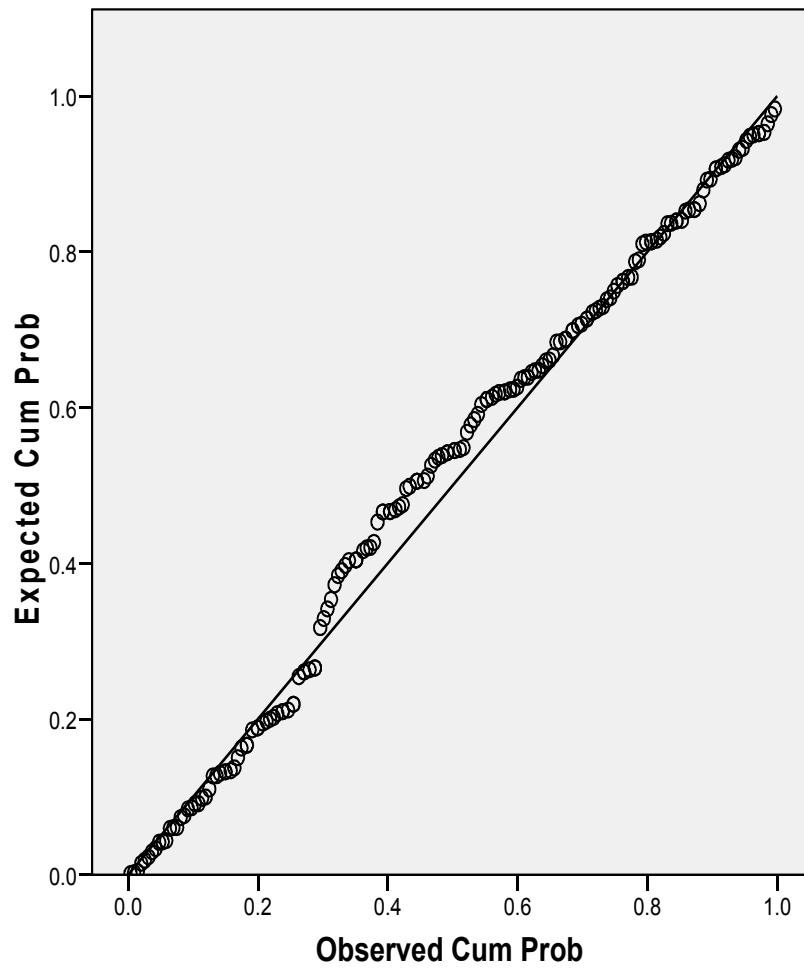


Fig 4.3 Probit plot for TAS.

Normal P-P Plot of vitC

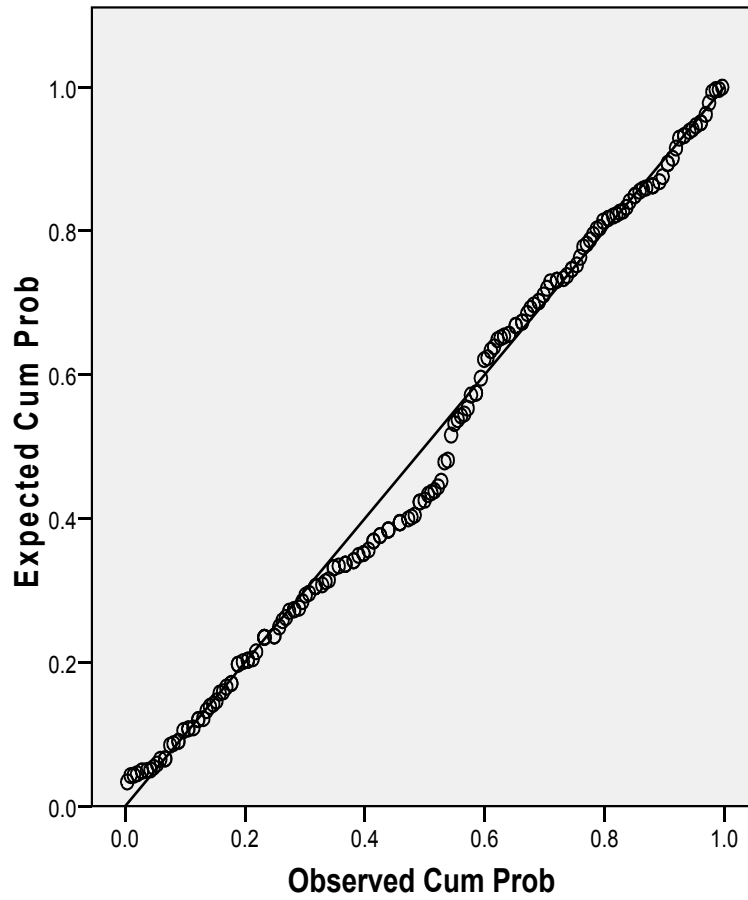


Fig 4.4 Probit plot for Vitamin C.

Normal P-P Plot of vitE

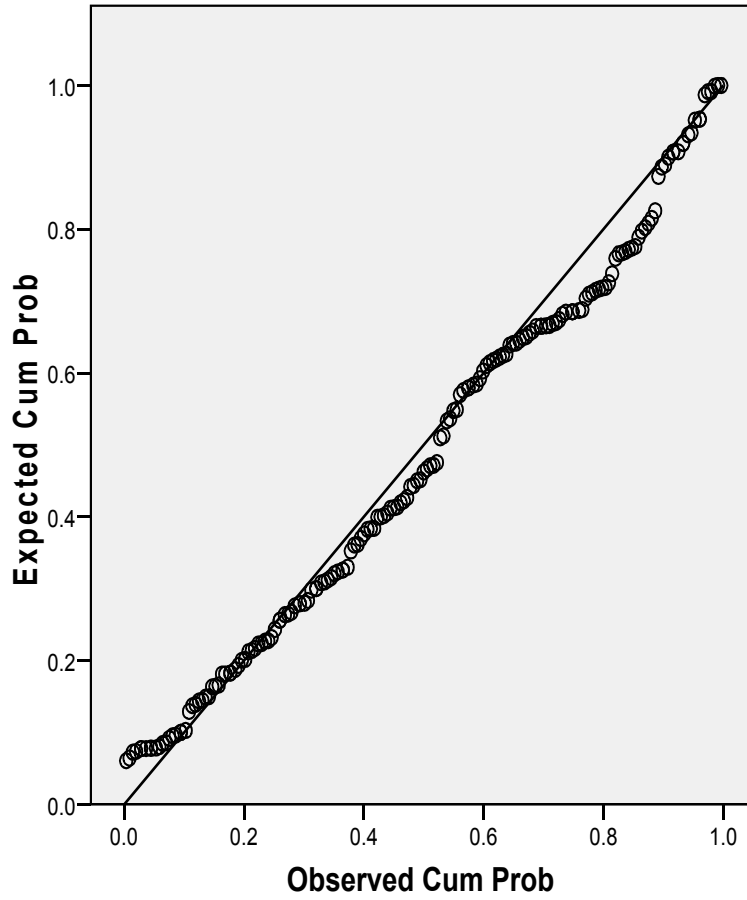


Fig 4.5 Probit plot for vitamin E.

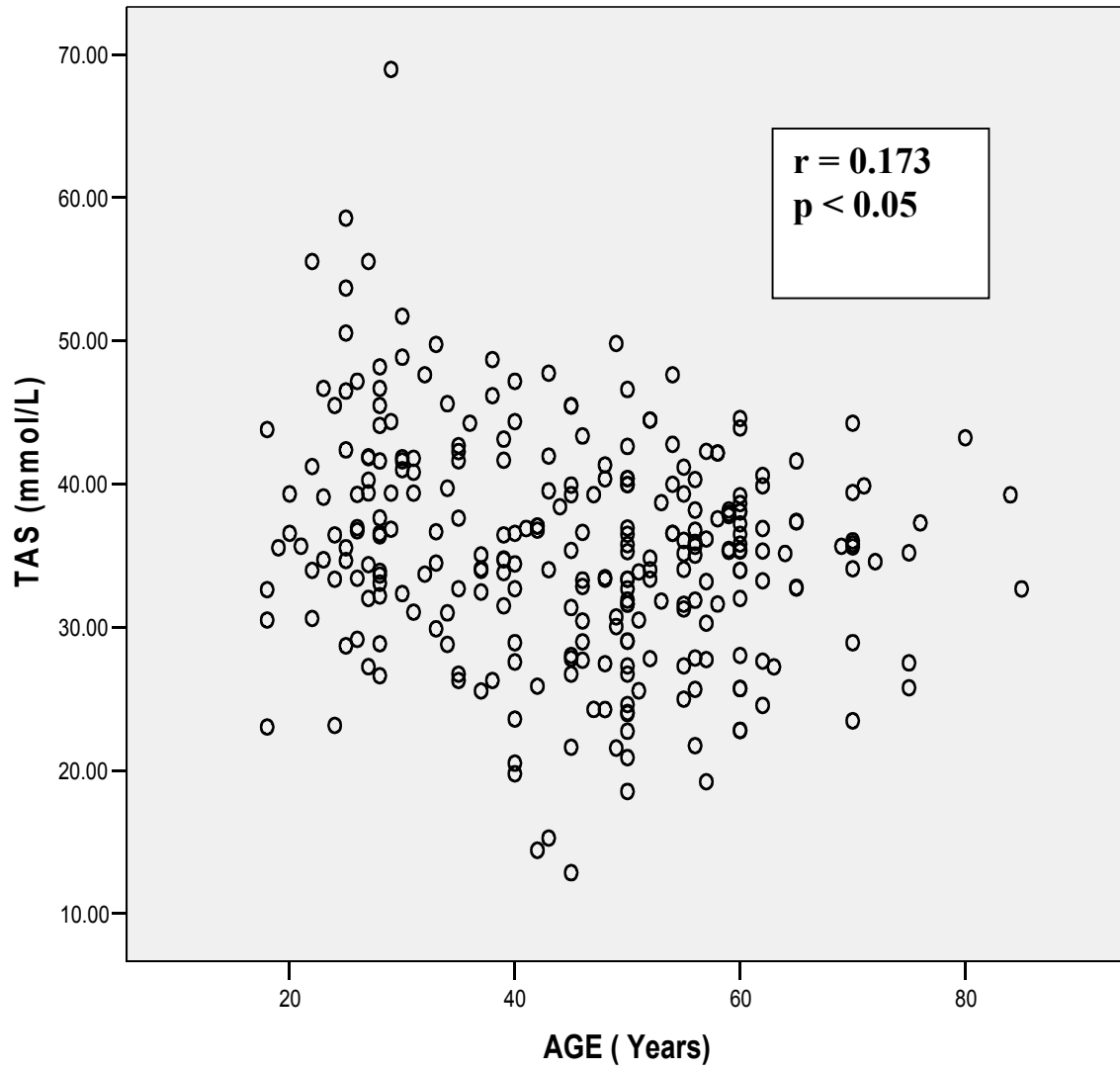
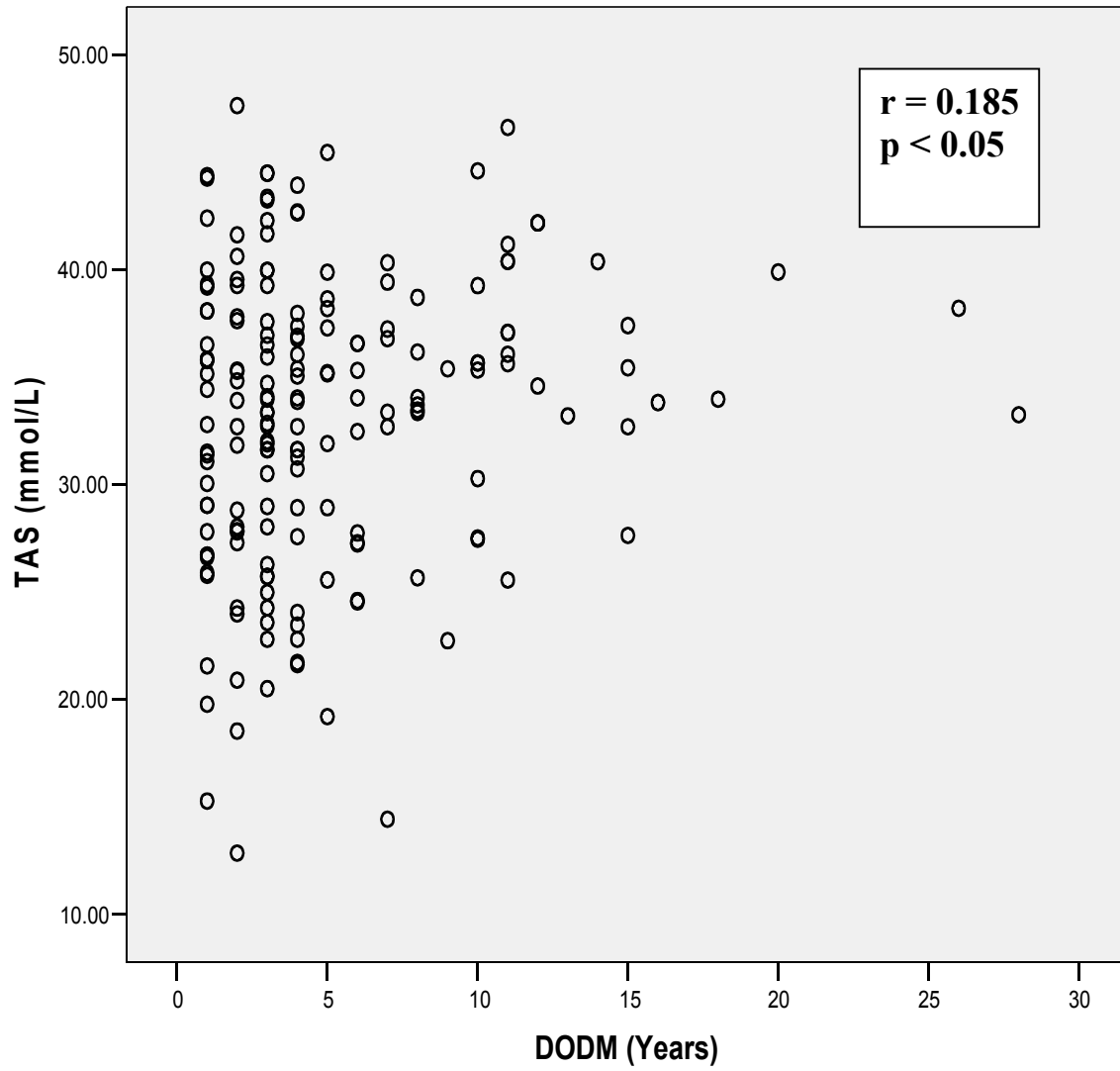


Fig 4.6: Relationship between TAS and age in diabetic patients.



CHAPTER FIVE

5.0 DISCUSSION

Diabetes mellitus usually begins gradually and progresses slowly. The early symptoms of untreated DM are related to elevated blood glucose levels and loss of glucose in the urine. The primary aim in the management of diabetic patients is to attain and sustain normoglycaemia. The problems are largely on the complications that could develop as a result of poor management of the disease. Management of diabetes mellitus is difficult due to poor levels of education and health care facilities in developing countries. Therefore, reported increase in the number of diabetic patients in Nigeria has been of great concern (Aliyu *et al*, 2005). The present study examined the serum levels of TAS, vitamins C and E as well as other biochemical analytes including FBG and GHbA_{1c} in diabetic patients and control subjects. Clinical parameters such as age, body mass index (BMI), duration of diabetes mellitus (DODM), systolic blood pressure (SBP) and diastolic blood pressure (DBP) were also studied.

The mean values of FBG and GHbA_{1c} were significantly higher in diabetic patients than in controls. However, 49.2 % were in poor glucose control and 67.4 % were in poor GHbA_{1c} control. This is consistent with earlier findings of Aliyu *et al* (2005) and Benrebai *et al* (2008), who found

significantly higher levels of serum FBG and GHbA_{1c} in diabetic patients. FBG levels significantly correlated with GHbA_{1c}. This is in agreement with the study of Awojobi *et al* (1991) who reported that there is a direct correlation between GHbA_{1c} and blood glucose levels. Higher percentage of patients with poor GHbA_{1c} control in this current study is suggestive of the reliability of GHbA_{1c} since it gives a better view of what is happening over time.

In the present study, TAS, which is a measure of antioxidant enzymes, vitamins and minerals status of diabetic patients, was significantly lower in diabetic patients than in control subjects. This is in agreement with earlier findings of Ramakrishna and Jaikhani (2007) who reported significantly lower levels of serum TAS in type II diabetic patients than in controls. Benrebai *et al* (2008) reported a very drastic decrease in serum TAS in diabetic patients than control subjects. Also, Akinosun and Bolajoko (2007) in Ibadan reported decrease in serum TAS in type II diabetic patients than controls. Similarly, in Brazil, a study revealed lower plasma antioxidant activity in diabetic patients than in healthy individuals (Medina *et al*, 2007). Studies revealed that TAS in type II DM is low and this could be attributed to low levels of vitamins C and E or other micro-nutrients in the blood (Lai, 2008). The decrease could be as a

result of poor glycaemic control probably because of poor compliance rate of the diabetic patients.

Total antioxidant status represents the extrinsic (micronutrients) trace elements, vitamins and intrinsic factors including group of organic antioxidants such as enzymes-catalase, glutathione peroxidase, superoxide dismutase and non- enzymatic anti- oxidants (GSH) and others like flavonoids, bilirubin and uric acid (Benerebai *et al*, 2008). Low level of TAS in diabetic patients could be as a result of decrease levels of antioxidant vitamins, enzymes and minerals.

In the present study, antioxidant vitamins C and E were significantly lower in diabetic patients than controls. Similar findings were reported by other workers (Sundaram *et al*, 1996; Maxwell *et al*, 1997; Nuttal *et al*, 1999; Ceriello *et al*, 2004; Sief and Youseef 2004; Aliyu *et al*, 2005; Afkahami-Ardekan and Shojoeldiny-Adekami 2007; Ramakrishna and Jailkhani 2007; Benrebai *et al*, 2008). They noted a significant decrease of vitamins C and E in diabetic patients when compared with controls. The low levels of these antioxidant vitamins may account for the low level of TAS seen in the present study. Vitamins C and E are major contributors to TAS (Nwose, 2009). However, Sirivatsan *et al* (2009) in Iran noted similar values of vitamin C and E in patients and controls. However, vitamin C and E were found to be non significantly correlated with GHbA_{1c}.

This is in agreement with the findings of Dogun and Ajala, (2005) that reported non significant correlation between vitamins C and E with the levels of FBG and GHbA_{1c}. On the other hand, Sawant *et al*, (2007), discovered highly significant inverse correlation between vitamin C and GHbA_{1c} .

Vitamin C lowers sorbitol level, which is harmful to the eyes and kidneys in patients with DM. Besides, it decreases protein loss through the urine and improves glucose tolerance in type II DM. In the present study, the decrease in the level of vitamin C could be due to the fact that it functions as an important component of cellular defense against oxygen toxicity and lipid peroxidation caused by free radicals. This indicates that poor diabetic control is associated with reduced serum antioxidant activities which were revealed in significantly lower mean levels of vitamin E in diabetic patients with poor glycaemic control.

In diabetic patients, there is always increased loss of water soluble vitamin C in urine. There could also be impaired transport and dietary deficiency as well as increase demand of vitamin C in diabetic patients (Sawant *et al*, 2007). The increased demand is to remedy the increased oxidative stress. It has been discovered that vitamin C is involved in the regeneration of vitamin E (Sirivatsan *et al*, 2009). These may be a contributing factor to the decrease in the levels of vitamin C observed in the diabetic patients of the present study. The results of the present study indicated negative correlations between GHbA_{1c} and

each of the antioxidant vitamins. Similar study by Aliyu *et al* (2005) and Sawant *et al* (2007) revealed that mean blood glucose and GHbA_{1c} levels correlated negatively with vitamins C and E.

The current study revealed that, mean values of vitamin E was significantly higher in those with good glycaemic control than in those with poor glycaemic control. Kaneto *et al*, (1999) and Gupta and Chari (2006) reported that improved glycaemic control leads to decrease in macrovascular diseases. This is in agreement with the studies of Ruhe and McDonald (2001), Aliyu *et al* (2005) and Chertow (2005) who established that there is a strong correlation between high glucose concentration and poor prognosis. Also a study in Iran shows that there is a significant correlation between total antioxidant status (TAS) and poor glycaemic control, hence the suggestion that measurement of TAS in DM could be used as an index of glycaemic control and development of diabetic complications (Rahbani – Nobar *et al*,1999). No significant correlation was found between TAS and glycaemic control in the present study. This indicates that patients in this environment could be having good management or compliance. Mean values of TAS in diabetic patients with good and poor glycaemic control were similar as observed in the present study. This could be due to the fact that only 2.2% of the studied diabetic population had TAS levels

below 15.7 mmol/L. Another reason for the above observation could be the fact that majority of the diabetic patients studied are in good glycaemic control.

The diabetic patients in the present study had complications like retinopathies and leg ulcerations. The mean value of vitamin E was discovered to be significantly higher in patients without complications than in those with complications. Similar findings were reported by Merzouk *et al* (2003) and Sawant *et al* (2007). This rise in antioxidant vitamin E could probably be due to an adaptive response to the pro-oxidant milieu of the diabetic state. Furthermore, Sirivatsan *et al* (2009) noticed an insignificant difference in vitamin C and E in diabetic patients with and without complication. This is in agreement with the present study. However, the involvement of vitamin C in the regeneration of vitamin E could be the reason for the low level of vitamin C seen in diabetic patients with complications than those without complications. Mean values of TAS in diabetic patients with complications were similar to those without complications as observed in the present study. The reason for the above observation could be the fact that majority (86.2%) of the diabetic patients studied were without complication.

The DODM among diabetic patients with complications was found to be significantly higher than in those without complications. Similarly, the DODM was found to be significantly correlated with TAS. It has been

discovered patients who had the disease for longer duration (above 15 years) with poor glycaemic control are prone to develop the long term complications (Kaneto *et al*, 1999; Rahbani – Nobar *et al*,1999; Papas, 2008). Several studies have shown that DM can worsen antioxidant status leading to deficiencies in some vitamins such as vitamins C and E which can aggravate several complications of DM (Kaneto *et al*, 1999; Rahbani – Nobar *et al*, 1999; Benrebai *et al*, 2008). From this present study, majority of the diabetic patients had the disease for 4 years this could be the reason why a greater number of the patients were without complication.

Since there are no reference values for antioxidant status in this environment, the reference values for TAS established in the current study may be employed in the biochemical assessment of our patients.

5.1 CONCLUSIONS

It can be concluded from the findings of the present study that:

1. the mean serum TAS, vitamins C and E levels were significantly lower in type II diabetic patients than in controls.
2. the mean FBG and GHbA_{1c} levels were significantly higher in diabetic patients than in control subjects.
3. the mean serum vitamin E level was significantly higher in patients with good glycaemic control than poor glycaemic control.

- 4. the mean serum vitamin E level was significantly higher in patients without complications than those with complications.**
- 5. the reference values for TAS ,vitamins C and E were 15.7 – 63.1 mmol/L, 0.2 – 7.0 mg/ml and 3.7- 119.3 g/L respectively.**

5.2 RECOMMENDATIONS

It can be recommended from the findings of the present study that :

- 1. measurement of TAS be included as one of the test menu in the evaluation of patients with type II diabetes mellitus.**
- 2. further study involving a larger sample size is suggested.**
- 3. the effects of drug therapy on the level of TAS be evaluated.**
- 4. measurement of other components of TAS is suggested.**

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APPENDIX I

CONSENT FORM

Serial number: _____

Age: _____

The research is designed to determine the levels of antioxidants in diabetic patients. You are free to participate or decline from the study.

The study will hopefully improve the present status of diabetic patients and that of others in future.

APPENDIX II

INFORMED WRITTEN CONSENT

I----- of ----- agreed to participate in this study of measurement of antioxidants in type II diabetic patients. The full procedure and probable benefits were explained to me. I made this consent willingly without being subjected to any pressure.

Participant's name----- signature-----

Witness name ----- signature-----

Researchers name ----- signature-----

APPENDIX III

QUESTIONNAIRE FOR THE STUDY OF EVALUATION OF TOTAL ANTIOXIDANT STATUS IN TYPE II DIABETIC PATIENTS.

1. Name
2. Hospital Number
3. Research Number
4. Age
5. Gender
6. Ethnic group
7. Address
8. Anthropometric measurement .
 - a. Weight (kg)
 - b. Height (m)
 - c. BMI
9. Duration of diabetes mellitus
10. Drugs regimen
11. Complications
12. Blood pressure: (a) systolic (b) diastolic
13. Laboratory investigation.
 - a. TAS
 - b. Vitamin C
 - c. Vitamin E
 - d. FBS
 - e. GHbA_{1c}