KWAME NKRUMAH UNIVERSITY OF SCIENCE & TECHNOLOGY,

KUMASI

SIALIC ACID, METABOLIC AND ANTHROPOMETRIC VARIABLES IN TYPE TWO DIABETIC PATIENTS.

A THESIS SUBMITTED IN FULFILLMENT

OF THE REQUIREMENTS FOR THE

DEGREE OF

MASTER OF PHILOSOPHY

(Chemical Pathology)

In the

Department of Molecular Medicine,

School of Medical Sciences

College of Health

By

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NOVEMBER, 2016

DECLARATION

The research work described in this thesis was carried out at the Department of Molecular Medicine-KNUST and Tema General Hospital between October, 2013 and March, 2015. This work has never been submitted for any other degree.

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ABSTRACT

Type 2 diabetes mellitus is associated with higher cardiovascular risk partly related to an increase in inflammatory parameters. There is however a gap in the fight to early detection of microvascular complications in type 2 diabetes in Ghana. The objective of this study was to determine the relationship between sialic acid with metabolic variables in Ghanaian type 2 diabetes with and without microvascular complications. This case-control study comprising of 150 type 2 diabetic patients and 50 agematched healthy individuals without diabetes were recruited. Fasting venous blood samples were collected and analyzed for total cholesterol, triglyceride, low-density lipoprotein, high-density lipoprotein, fasting glucose, glycated hemoglobin, sialic acid and C-reactive protein. Serum sialic acid levels were elevated in diabetic with retinopathy (230.68 ± 135.60mg/dl) followed by nephropathy (196.53 ± 61.19mg/dl) compared to diabetic patients with no complications (185.55 ± 75.69mg/dl) ($p=0.065$). Patients with type two diabetes had significantly higher C-reactive protein ($p=0.026$) and serum sialic acid ($p<0.0001$) compared to the non-diabetics. In the patients with diabetes, 14.7% were able to excellently control their blood glucose with 28.0% poorly controlling their glycaemia. Level of Serum sialic acid was higher in the patients with good control than those with poor control. However, C-reactive was higher in patients with poor control and lower in those with good control. The inflammatory markers showed no statistically significant difference on comparison. No statistically significant relationship ($P>0.05$) was established between the levels of serum sialic acid and blood glucose and glycated haemoglobin among patients with diabetes. C-reactive was inversely associated with blood glucose though not statistically significant, whereas it was directly associated with glycated. Serum sialic acid and C-reactive protein both showed inverse relationship with total cholesterol levels, triglycerides and low lipoprotein. However, a direct relationship between serum sialic acid and high density lipoprotein with no significance ($r= 0.018$, $P=0.825$) was observed. The main findings of this study is that raised serum sialic acid concentration is strongly related to the presence of

microvascular complications in type two diabetes particularly retinopathy and nephropathy. Again this study indicated direct association of glycated with elevation of serum sialic acid and Creactive protein. These may imply a significant relation between inflammation and glycemic control in people with established diabetes.

DEDICATION

I dedicate this work to my dear husband Mr George Larbi, my children Jessica, Amadea and Jeremy

ACKNOWLEDGEMENT

My first thanks goes to God for the grace, his traveling mercies and finally seeing me through.

I express my sincere gratitude to my supervisor, Dr. Christian Obirikorang Lecturer at the Department of Molecular Medicine, School of Medical Sciences, College of Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi for their valuable suggestions, direction, guidance and advice at all stages of this work. I am also grateful to Dr. Seth Amanquah a Lecturer at University of Ghana, Chemical Pathology Department for the help and support he gave me. I also thank my former medical director Dr Charity Sarpong and my current director Dr Opoku Adusei for allowing me carry out my project work at Tema General Hospital.

The advice and cooperation of the Dr Tsikata and nurses of the diabetic clinic and medical laboratory scientists of the Chemical pathology department of Tema General Hospital is very much appreciated. I also wish to thank the Tendosis family for their help and support. My sincere thanks also go to Hope Edem for the support he gave me with my work. I say a big thank you to my husband Mr George Larbi for the encouragement and financial support he gave me and my children Jessica, Amadea and Jeremy for their co-operation.

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ABBREVIATIONS

A1C/ HbA1C	Glycated haemoglobin
AACE	American Association of Clinical Endocrinologist
ADA	American Diabetes Association
AGEs	Advanced glycation endproducts
AIDS	Acquired immune deficiency syndrome
ARIC	Atherosclerosis Risk in Communities
BAI	Basal adiposity index
BMI	Body mass index
C1Q	Complement component
Camp	Cyclic adenosine monophosphate
CAT-I	Carnitine acyl transferase I
CHD	Coronary heart disease
CRP	C-reactive protein
CVD	Cardiovascular disease
CVD	Cardiovascular heart disease
DBP	Diastolic blood pressure
DKA	Diabetic ketoacidosis
DM	Diabetes Mellitus
EDTA	Ethylenediamine tetraacetic acid (sequestrine)
FBG	Fasting blood glucose
FFA	Free fatty acid
GDM	Gestational diabetes mellitus
HbA1C/GHb	Glycated haemoglobin
HDL	High density lipoprotein
HHS	Hyperosmolar hyperglycaemic state
HIV	Human immunodefiency virus
HRP	Horseradish peroxidase
IFG	Impaired fasting glycaemia
IGT	Impaired glucose tolerance
IL	Interleukins
IR	Insulin receptor
Kg/m^2	Kilogram per meter squared
LADA	Latent autoimmune diabetes of (in) adults

LDL	Low density lipoprotein
LSA	Lipid – bound sialic acid
mmHg	Millimeter mercury
mg/dl	Milligram per deciliter
mmol/l	Millimol per liter
NCEP ATP III	National Cholesterol Education Programme Adult Treatment Panel III
NDSR	National Diabetes Statistics Report
NH	Ammonia
Neu5Ac /NANA	N-acetylneuramic acid
OGTT	Oral glucose tolerance test
PAI-1	Plasma plasminogen activator inhibitor
PEPCK	Pyruvate carboxylase, phosphoenolpyruvic carboxykinase *
PRR	Pattern recognition receptor
SAA	Serum amyloid A
SSA	Serum sialic acid
SBP	Systolic blood pressure
TC	Total cholesterol
TG	Triglycerides
TNF-α	Tumor necrosis factor-alpha*
US	United states of America*
VAI	Visceral adiposity index
VLDL	Very low density lipoprotein
W.H.O	World health organization
WC	Waist circumference
WHR	Waist to hip ratio

Chapter 1

INTRODUCTION

1.1 GENERAL INTRODUCTION

Diabetes mellitus is a metabolic disorder mostly characterized by persistent hyperglycaemia. It is associated with usual symptoms such as frequent urination, increased thirst and increased hunger (Kumar *et al.*, 2005). Unlike type 1 diabetes mellitus which is occurs as a result of the loss of insulin production, type 2 diabetes is characterized by insulin resistance (Haffner *et al.*, 2002).

Approximately, 285 million people globally had diabetes as of 2010, with 90% of the cases being type 2 diabetes (Hedley *et al.*, 2004). By 2030, this number is expected to double from about 2.8% to 4.4% (Wild *et al.*, 2004). Type 2 diabetes is mostly common in the developed world but is expected to increase in Sub-Saharan Africa, Middle Eastern Crescent, India and Asia (Wild *et al.*, 2004). In Ghana, the crude prevalence and age-adjusted prevalence were observed to be 6.3% and 6.4% respectively (Amoah *et al.*, 2002). This rate is higher than that of the world prevalence rate and raises concern about the implications of diabetes and its toll on the country.

The debilitating aspects of diabetes are the numerous complications which includes diabetic retinopathy, nephropathy, cardiovascular complications and peripheral neuropathy. However, the progression and severity of these complications are directly associated with the duration of the disease and management strategies (Nayak & Bhaktha, 2005).

Diabetes is associated with increased risk of cardiovascular diseases due to metabolic factors such as an dyslipidaemia, obesity, hypertension and physical inactivity (Nayak *et al.*, 2005). Inflammation is associated with the development and progression of diabetes mellitus in

populations with the risk of atherosclerosis (Haffner *et al.*, 2002; Duncan *et al.*, 2003). This link however, was only in white non-smokers but not among African Americans and smokers, indicating that lifestyle and race could influence this association (Pradhan *et al.*, 2001).

Sialic acid is a component of cell membranes. Elevated levels in plasma or serum indicate excessive damage to vascular tissues of the cell membrane as well as organs such as retina of the eyes, kidneys, heart and brain leading to complications (Nayak & Roberts, 2006; Yarema, 2006). Glycoproteins form a major part of proteins of the immune response which have sialic acid as the terminal sugar on their oligosaccharide chain (Pickup, 2004). This makes sialic acid a useful marker of acute phase response. Its relation with inflammation means it could serve as a diagnostic and predictive tool for future diabetic complications.

The main aim of the current study therefore, was to determine whether in Ghanaian type 2 diabetics, there are higher sialic acid levels, which can serve as a predictive marker of microvascular complications.

1.2 PROBLEM STATEMENT

Diabetes comes with numerous complications including retinopathy, nephropathy, cardiovascular complications, and peripheral neuropathy. These complications have become inevitable as most cases are identified only after onset. Sialic acid which is a component of cell membranes is elevated in cell membrane damage of vascular tissue (Yarema, 2006). There is the need to identify early markers, which will not only monitor disease progression and prognosis soon after diagnosis, but also predict the onset of microvascular complications.

1.3 AIM

This study evaluated the levels of sialic acid and its association with lipid profile and anthropometry among type 2 diabetics with or without microvascular complication in the Tema Metropolis, Ghana.

1.3.1 Specific objectives

1. To determine the inflammatory response in type 2 diabetic participants with and without microvascular complication using sialic acid and C-reactive protein.
2. To determine the changes in lipid profile (Total cholesterol, triglyceride, high density lipoprotein) and anthropometric variables (Body mass index, waist circumference, body adiposity index, and vascular adiposity index) in type 2 diabetics with and without microvascular complications.
3. To determine the association between sialic acid levels and lipid profile and anthropometric measures
4. To determine the predictive ability of serum sialic acid to microvascular complications.

1.4 JUSTIFICATION

Ghana is a developing country, with scarce resources and a diabetic mellitus prevalence rate higher than that of the world (Amoah *et al.*, 2002) Thus the implications of diabetes mellitus and its associated complications is a major public health concern. Hence markers for early prediction and detection are of importance in managing life expectancy associated with diabetes.

The development and severity of these complications are dependent on the duration of the disease and how early it is detected and managed (Nayak *et al.*, 2005). There is a gap in the fight to early detection of microvascular complications in type 2 diabetes in Ghana.

Assessment of cardiovascular, nephropathic and retinopathic risk among type 2 diabetics here in would enhance knowledge and also provide enable medical practitioners resources to better manage their diabetic patients.

1.5 STUDY HYPOTHESIS

1. Serum sialic acid levels are higher in type 2 diabetes mellitus associated complication compared to healthy people.

Chapter 2

LITERETURE REVIEW

2.1 DIABETES

Diabetes Mellitus (DM) is a metabolic disorder characterized by chronic high blood sugar, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both (W.H.O, 2006). Diabetes mellitus affects over 230 million people globally and it's expected to increase to 350 million by 2025. Of all the major types of diabetes, type 2 diabetes mellitus account for 90% of all forms of diabetes (Kumar *et al.*, 2013). The chronic high blood sugar in diabetes is related with significant longterm sequelae, particularly dysfunction and failure of several organs such as kidneys, eyes, nerves, heart and blood vessels (Nayak & Roberts, 2006).

2.1.1 Global State of Diabetes

Globally, diabetes is gradually achieving epidemic proportions. The International Diabetes Federation estimated in 2003, that approximately 194 million people around the world had diabetes. This figure is expected to rise to 333 million by 2025, which means that 6.3% of the world's population will be living with diabetes (W.H.O, 2006).

About 37% of U.S. adults who were 20 year and above had prediabetes bases on HbAIc and glucose estimation from 2009−2012. According to the American Diabetes Association, 2014 about 208,000 people below 20 years representing 0.25% have been diagnosed of type 1 or type 2 diabetes (American Diabetes Association, 2014).

Furthermore, India and China are a classical example of a dramatic rise in diabetes prevalence. India is the largest diabetic population in the world with an estimated prevalence of 8% of the total population of 35 million while in China about 2.7% of the adult population is affected with type 2 diabetes.

2.1.2 Diabetes in Africa

Diabetes was previously thought to be uncommon or undocumented in rural Africa, but over the past few decades it has emerged as an imperative non-communicable disease in sub-Saharan Africa (McLarty *et al.*, 1990; Levitt, 2008; Motala *et al.*, 2008). Blacks are 1.7 times more likely to develop diabetes than whites according to the Center for Disease Control and Prevention (A.D.A, 2014). Type 2 diabetes however makes up about 90% of the cases (Hedley *et al.*, 2004; Wild *et al.*, 2004). The crude prevalence rate and age-adjusted prevalence was 6.3% and 6.4% respectively among type 2 diabetes in Ghana (Amoah *et al.*, 2002).

2.2 CLASSIFICATION OF DIABETES MELLITUS

The American Diabetes Association (American Diabetes Association, 2014) classify diabetes into four clinical classes*:* Firstly, Type 1 diabetes (results from β-cell destruction, usually leading to absolute insulin deficiency) Secondly, Type 2 diabetes (results from a progressive insulin secretory defect on the background of insulin resistance). Thirdly, Gestational diabetes mellitus (GDM) (diabetes diagnosed during pregnancy that is not clearly overt diabetes**)"** Fourthly, Other specific types of diabetes due to other causes, e.g., genetic defects in β-cell function, genetic defects in insulin action, diseases of the exocrine pancreas (such as cystic fibrosis), and drug- or chemical-induced (such as in the treatment of HIV/AIDS or after organ transplantation)

2.3 CAUSES OF DIABETES

2.3.1 Cause associated with Type 1 diabetes

Type 1 diabetes was earlier known as insulin dependent diabetes mellitus or juvenileonset diabetes. The peak age for diagnosis is in the middle teens though the disease onset can also occur at any age (Cooke, 2008). In genetically susceptible people

the onset of diabetes can be triggered by one or more environmental factors, such as a viral infection and diet. Meanwhile, this condition is partly inherited. Type 1 diabetes occurs when the beta cells in the pancreas are damaged. This destruction is instigated by the body's immune system which partially or fully eliminates the production and secretion of insulin (McLarty *et al.*, 1990; Goldstein *et al.*, 2004).

Type 1 diabetes accounts for approximately 5% of all diagnosed cases of diabetes in adults' population. The individual with type one is mostly manages by injecting insulin. Contrast to type 2 diabetes, the onset of type 1 diabetes is not related to lifestyle characteristics. Irregular and unpredictable hyperglycemia regularly occurs with ketosis and occasionally with serious hypoglycemia mostly follows the onset of Type 1 diabetes. Other complications such as infection, impaired counter regulatory response to hypoglycemia, gastroparesis, and Addison's disease occur in 1% to 2% of individuals with type 1 diabetes (Levitt, 2008; Motala, 2010).

2.3.2 Causes associated with Type 2 diabetes

Type 2 diabetes also known as non–insulin dependent diabetes mellitus usually occurs in adult population unlike type 1 diabetes. It account for 90% to 95% of all diagnosed diabetes cases. It is characterised by insulin resistance where primary cells within the fat tissue, fats and muscle do not properly use insulin (Cooke, 2008). The beta cells in the pancreas steadily lose the capacity to produce enough numbers of the hormone as the need for insulin arises. The role of beta cell dysfunction as constrast to insulin resistance varies among individuals. Some individuals may predominantly have insulin resistance or a minor defect in insulin secretion while others may have slight insulin resistance or primarily lack insulin secretion (Goldstein *et al.*, 2004)

There are several factors such as family history of diabetes, poor nutritional status, old age, impaired glucose metabolism, physical inactivity, obesity, history of gestational diabetes and race are commonly related with type 2 diabetes. Obesity accounts for 30% of cases among Chinese and Japanese descent, 60-80% of European and African descent, and 100% of Pima Indians and Pacific Islanders. Those who do not have high body weight often have a high waist–hip ratio (Kuzuya & Matsuda, 1997).

Dietary factors such as excessive consumption of cocktails, high carbohydrate and fatty diets are associated with an increased risk (Malik *et al.*, 2010). Trans fatty Acids and saturated fats increase the risk while polyunsaturated and monounsaturated fat decrease the risk of type 2 diabetes (Risérus *et al.*, 2009). Consumption of high carbohydrates diet such as white rice plays a key role in increasing risk of diabetes mellitus (Hu & Stampfer, 2003). Physical inactivity is believed to account for 7% (Lee *et al*., 2003).

2.3.3 Cause associated with Gestational diabetes

Like Type 2 diabetes, gestational diabetes mellitus (GDM) involves a combination of relatively insufficient insulin secretion and sensitivity. It is mainly associated pregnancy and it account for 2–5% of all cases and resolve postpartum. Gestational diabetes is can be completely treated and managed. However, it requires constant monitoring and care during this period. Approximately 20–50% of GDM women tend to develop type 2 diabetes later in life (Kim *et al.*, 2002).

GDM is associated with several complications and adverse outcomes of pregnancy such as high birth weight, skeletal muscle malformations, and congenital cardiac and central nervous system anomalies. Elevated fetal insulin may hinder fetal surfactant production culminating in respiratory distress syndrome, hyperbilirubinemia, poor placental perfusion due to vascular impairment, caesarean section (Clausen *et al.*, 2008). Induction

of labour may be associated with reduced placental perfusion while caesarean section are mostly performed if there is massive fetal distress or macrosomia, such as shoulder dystocia (Lawrence *et al.*, 2008).

Table 2.1 Comparison of Type 1 and 2 Diabetes

Feature	Type 1 diabetes	Type 2 diabetes
Onset	Sudden	Gradual
Age at onset	Mostly in children	Mostly in adults
Body habitus	Thin or normal	Often obese
Ketoacidosis	Common	Rare
Autoantibodies	Usually present	Absent
Endogenous insulin	Low or absent	Normal, decreased or increased
Concordance in identical twins	50%	90%

2.4 SIGNS AND SYMPTOMS OF DIABETES

CLASSICAL SIGNS & SYMPTOMS

It includes polydypsia, polyphagia, polyuria, pruritis, weakness & fatigue. (More common on type 1) occur in varying degree in type 2 DM.

Type 1 DM may associated with Weight loss, Ketoacidosis

Restlessness, irritability & apathy may become evident.

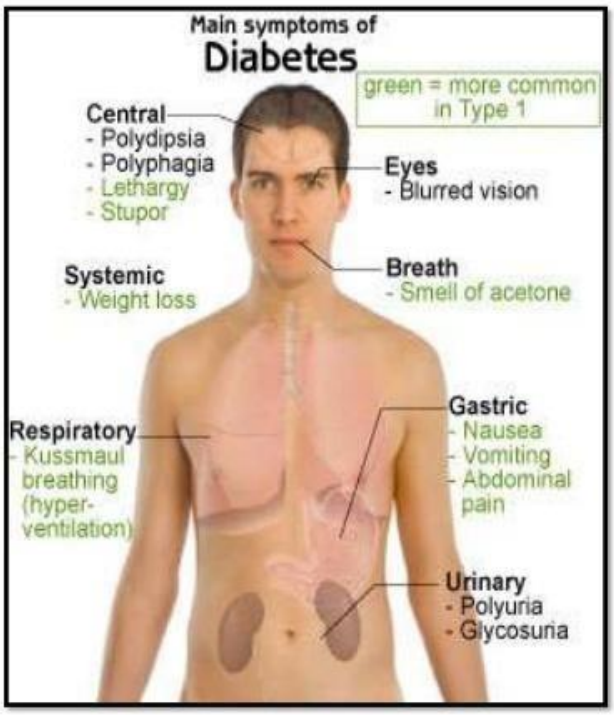


Figure 2. 1 Signs and symptoms of Diabetes (Source: Cooke, 2008)

The major symptom of diabetes include weight loss, frequent urination, increased thirst and increased hunger. Symptoms may progress quickly for weeks or months in type 1 diabetes while they usually develop slowly or may be subtle in type 2 diabetes (Cooke, 2008).

Persistent hyperglycaemia can result in blurred vision due to glucose absorption in the lens of the eye. Retinopathy is common complication associated with most diabetics.
Diabetic dermadromes, represent a number of skin rashes that can occur in diabetes (Pickup, 2004).

2.5 DIAGNOSIS OF DIABETES MELLITUS

The clinical diagnosis of diabetes mellitus is mostly depends as recurrent infections, drowsiness and coma, polydipsia, unexplained weight loss, and in severe cases and polyuria (Umpierrez *et al.*, 1997). There is usually the presence of Hyperglycosuria.
Plasma glucose concentration ≥11.1 mmol/L and venous whole blood glucose concentration ≥10.0 mmol/L establishes the laboratory diagnosis of DM. Non-DM individual reports with random blood glucose below 5.5 mmol/L with venous whole blood below 4.4 mmol/L (American Diabetes Association, 2005). An oral glucose tolerance test (OGTT) is needed to establish diagnostic status if casual blood glucose between ≥5.5 and <11.1 mmol/L for venous plasma, ≥4.4 and <10.0 mmol/L for venous whole blood and fasting blood glucose levels are above the upper reference limit but below those which establish the diagnosis of diabetes mellitus. It is sufficient to measure the blood glucose values whilst fasting and at 2 hours after a 75g oral glucose load when an OGTT is performed.

Fasting venous plasma glucose concentration of 6.1 mmol/L or greater but less than 7.0 mmol/L and if a 2-hour postprandial is measured and fasting venous plasma glucose levels of less than 7.8 mmol/L it is considered as Impaired Fasting Glycaemia (IFG). However, a fasting venous plasma 2-hour post glucose concentration of 7.8 mmol/L or greater but less than 11.1mmol/L and if a fasting glucose is measured and a fasting venous plasma glucose concentration of less than 7.0 mmol/L the patient is considered as Impaired Glucose Tolerant. (IGT).

The diagnosis of DM must always be established by running multiple test per day unless there is an undeniable hyperglycaemia with acute metabolic decompensation or obvious symptoms as recommended by the expect committee (Alberti & Zimmet,
1998)

Glucose levels must not be determined on serum unless red cells are immediately separated else glycolysis can result in wrong glucose concentrations. Glycolysis may not be completed stop by glucose and so if whole blood is used the sample should be kept at $0 - 4\ ^{0}C$, or analyzed immediately and if plasma is used the blood must be centrifuged immediately (Alberti *et al.*, 1998).

Aside the previous diagnostic tools, glycated haemoglobin (HbA1c) seem to reflect a better glycaemic event because it gives an average glycaemia over the preceding 2─3 months. Even though in certain instances HbA1c gives almost equal sensitivity and specificity to glucose measurement, it is mostly underutilized in low resource constraint countries (McCance *et al.*, 1994). HbA1c is measure of risk for the development of micro- and macrovascular complications (Crowther *et al.*, 2005; Krolewski *et al.*, 2014) and it is currently considered the best index of metabolic control for diabetic patients in clinical settings (Goldstein *et al.*, 2004; Nathan *et al.*, 2005). For large sample size studies of glucose intolerance and diabetes, individuals have been categorized by their

blood glucose concentration estimated after 2 hours after a 75g oral glucose load or and an overnight fast. Epidemiological studies or diagnostic screening have in the past been restricted to the 2-hour values only since, it may be difficult to be sure of the fasting state, and because of the strong correlation between fasting and 2-hour values. Although this remains the single best choice, if for logistical or economic reasons OGTT is not performed, the fasting plasma glucose may be used for epidemiological purposes (Alberti *et al.*, 1998). A standard OGTT must be performed after 12 – 14 hours overnight fasting by giving 75g anhydrous glucose in 250-300ml water to determine if gestational diabetes mellitus is present in pregnant women and plasma glucose is measured at fasting and 2 hours after glucose intake. The pregnant woman should be reclassified as either having diabetes mellitus, IGT, or normal glucose tolerance based on the results of a 75g OGTT six weeks or more after delivery (Alberti *et al.*, 1998).

2.6 DIABETES MELLITUS AND ASSOCIATED COMPLICATIONS

The major long-term complications of diabetes relate to destruction to blood vessels. The risk of cardiovascular disease and 75% of mortality in diabetics are usually due to coronary artery disease (Sarwar *et al.*, 2010; O'Gara *et al.*, 2013). Damage to the eyes, kidneys, and nerves constitute the major forms of microvascular complications of diabetes.

Diabetic Retinopathy is caused by destruction to the blood vessels in the retina, and can result in gradual loss of sight and potentially blindness. Diabetic Nephropathy, can lead to urine protein loss, tissue scarring and eventually chronic kidney disease, and may sometimes require dialysis or kidney transplant. Diabetic Neuropathy includes symptoms such as tingling, numbness, painful muscle wasting, weakness and altered pain sensation which can results in destruction to the skin. Diabetes foot is one major

complication of diabetes and may be difficult to treat sometimes. Additionally, proximal diabetic Neuropathy causes painful muscle wasting and weakness.

(Christensen *et al.*, 2009).

2.7 METABOLIC EFFECTS OF INSULIN AND DIABETES MELLITUS

The principal hormone responsible for the regulation of glucose metabolism is insulin. Insulin is produced by the β-cells of the islets of Langerhans of the pancreas as a precursor pro-insulin, which is form insulin and C-peptide (Domanski & Proschan, 2004). Two polypeptide chains the A and the B forms 21 and 30 amino acids respectively. These chains are allied together by A7 to B7 and A20 to B19 inter-chain disulphide bridges and intra-chain disulphide bridge in the A chain which attaches residues 6 and 11. Insulin secretion is primarily regulated by plasma glucose concentration. Insulin regulate the uptake and utilization of glucose in peripheral tissues through the glucose transporter and may also inhibit of hepatic gluconeogenesis and glycogenolysis using hyperglycaemic hormones such as cortisol, adrenaline, growth hormone, glucagon and thyroxine (Kahn, 2003). Individual with hypopituitarism and type 1 diabetes may have reduced insulin concentration.

Condition such as insulinoma, obesity, Cushing's syndrome, type 2 diabetes mellitus and acromegaly are usually associated with elevated insulin concentrations. Insulin signaling are necessary for normal growth development and normal homeostasis of carbohydrate protein and lipid metabolism. Activation of the insulin receptor (IR) has been the principal focus of a several authors and studies for decades.(Ref.) Several prospective studies (Ferrannini, 1998; Weyer *et al.*, 2001) in numerous inhabitants showed that insulin resistance and insulin secretory dysfunction is a better predictor for the development of type 2 diabetes and this area has attracted many research focus and therapeutic targets. The pathophysiology of insulin resistance associated with type 2

diabetes mellitus and obesity can be better understood by understanding the signaling pathways involved in insulin action. Identification of biomolecules and events target pathways could serve as effective therapeutic agents for treatment.

2.8 METABOLIC COMPLICATIONS OF DIABETES MELLITUS

The two most common and dangerous types of acute metabolic complications of diabetes mellitus are hyperosmolar hyperglycaemic state (HHS) and Diabetic ketoacidosis (DKA) because they occur in both type 1 and type 2 diabetes. The mortality rate DKA and HHS are is <5% and 15% respectively (Hamblin *et al.*, 1989; Basu *et al.*, 1992). In the a comatose state, advanced age and hypotension, the prognosis of both conditions is significantly worsened (Malone *et al.*, 1992). The main biochemical picture of DKA are ketonaemia, acidaemia and hyperglycaemia. Unlike HHS where severe hyperglycaemia and hyperosmolality are major determinants of the condition, hyperglycaemia in DKA is unpredictable and may not be an independent factors of the severity of DKA. Ketosis is a major component of HHS and may often occur without coma. Mental status in DKA and HHS has been linked with osmolality (Ennis *et al.*, 1994; Umpierrez *et al.*, 1997).

The basic underlying mechanism for DKA and HHS is a reduction in the net effective concentration of circulating insulin even though the pathogenesis of DKA is better understood than that of HHS (Polonsky *et al.*, 1994), as well as corresponding increase in of counter regulatory stress hormones. Both conditions are due to impaired carbohydrate regulation that can as well occur in diabetes mellitus (Umpierrez *et al.*, 1997). The deficiency in insulin can be absolute or insufficient comparative to an elevated counter regulatory hormones in DKA patients. In HHS insulin secretion does not control hyperglycaemia but reduces ketosis and may results into severe dehydration and renal insufficiency and a subsequent decreased excretion of glucose (Ennis *et al.*,

1994). The above factors coupled with stressful condition and inadequate fluid intake contributes to severe hyperosmolality, the hallmark of HHS.

Infections such as pneumonia and urinary tract infections accounts for 30 to 50% of infection in DM and also form a major factor for the development of DKA or HHS (Basu *et al.*, 1992).

Conditions such as pulmonary embolism, trauma, myocardial infarction, alcohol abuse and pancreatitis which can occur both in type 1 and type 2 diabetes also precipitate DKA and HHS (Nathan *et al.*, 2005).

Extreme use of diuretics in the elderly and drugs such as pentamidine, sympathomimetic agents, corticosteroids and α- and β-adrenergic blockers in DM may also precipitate the development of DKA and HHS.

Other precipitating factors for recurrent ketoacidosis are psychological factors and poor compliance. Psychological problems complicated by eating disorders may account for up to 20% of cases of recurrent ketoacidosis in young female patients with type 1 diabetes, (Polonsky *et al.*, 1994; Rydall *et al.*, 1997).

2.9 METABOLISM OF CARBOHYDRATE IN DIABETES

The major stage of developing hyperglycemia includes accelerated glycogenolysis, increased gluconeogenesis and impaired glucose utilization by peripheral tissues (Luzi *et al., 1988*; Vaag *et al., 1992*). High production of glucose by the hepatic cells results from the high availability of gluconeogenic precursors. Gluconeogenic precursors such as the lactate occurs as a result of high muscle glycogenolysis and glycerol occurs as a result of high lipolysis, and amino acids alanine and glutamine occurs as a result of proteolysis and reduced protein synthesis. High glucose production by the kidney and liver represents the major pathogenic disturbance responsible for hyperglycaemia,

(Foster & McGarry, 1983; Hue, 1987; Exton, 1987; Siperstein, 1992). Though the extensive work in biochemical mechanisms for gluconeogenesis are well established, the molecular basis and the role of counter regulatory hormones in DKA are inconsistent. There are scarcity of studies in the attempt to determine the association between the increase in the level of counter regulatory hormones and the metabolic alterations in DKA (Schade & Eaton, 1980). Previous studies (Alberti *et al.*, 1975; Luzi *et al.*, 1988) showed that combination of increased catecholamines and a decreased level of free insulin may initiate the event in hydrated individuals. Whereas glucose levels increase with simultaneous increases in serum potassium, vomiting or other stress situations, ketosis is usually mild in the absence of dehydration (Kitabchi, 1989). In addition, the excessive decrease in the insulin also culminates in decreased glycogen synthase.

The final step in glucose production occurs by hydrolysis of glucose-6-phoshate to glucose, which is catalyzed by another rate-limiting enzyme of gluconeogenesis, hepatic glucose-6-phosphatase, which is stimulated by increased catabolic hormones and decreased insulin levels. Lactate, alanine in the liver, glycerol, and glutamine in the kidney form the major substrates for gluconeogenesis. The process of excess proteolysis and decreased protein synthesis which occurs as a result of increased catabolic hormones and decreased insulin forms alanine and glutamine (Meyer *et al.*, 2000; Butte, 2000). Though increase hepatic gluconeogenesis is the key mechanism of hyperglycaemia in severe ketoacidosis, recent studies have shown that a significant portion of gluconeogenesis may be produced through the kidney (Meyer *et al.*, 1998).

2.10 KETONE AND LIPID METABOLISM

The combination of insulin deficiency and increased concentrations of counter regulatory hormones increases the production of ketones in DKA from phosphorylation and activation of hormone-sensitive lipase in adipose tissue (McGarry, 1979; Jensen *et*

al., 1989; Nurjhan *et al.*, 1992). A breakdown of triglyceride into glycerol and free fatty acids (FFAs) occurs via the increase activity of tissue lipase. The massive release of FFAs assumes pathophysiological predominance in the liver although glycerol is used as a substrate for gluconeogenesis in the liver and the kidney, where the FFAs serve as precursors of the ketoacids in DKA (McGarry, 1979; DeFronzo *et al.*, 1994). Increased level of glucagon in DKA reduces the hepatic levels of malonyl-CoA by obstructing the metabolism of pyruvate to acetyl-coA through inhibition of acetyl-CoA carboxylase (Gerich *et al.*, 1976; McGarry, 1979; Nurjhan *et al.*, 1992). Malonyl-CoA obstructs the rate limiting enzyme for transesterification of fatty acyl-CoA to fatty acyl-carnitine and regulate oxidation of fatty acids to ketone bodies. CAT-I is essential for movement of FFA into the mitochondria where fatty acid oxidation occurs. High CAT-I and fatty acylCoA activity in DKA results to high ketogenesis in DKA (McGarry *et al.*, 1989; Zammit, 1994). There is evidence of decrease clearance of ketones in patients with DKA (Reichard *et al.*, 1986; Balasse & Fery, 1989). Reduced ketone may be due to reduced insulin concentration, decrease glucose utilization by peripheral tissues and increased glucocorticoid level (Nosadini *et al.*, 1989). Epinephrine has a significant effect to increase lipolysis in adipocytes in vitro while in vivo, it increase level of FFAs in the presence of insulin deficiency (Avagaro *et al.*, 1993).

2.11 METABOLIC SYNDROME AND ITS RELATIONSHIP WITH DIABETES CRITERIA FOR CLINICAL DIAGNOSIS OF THE METABOLIC SYNDROME

There are four different criteria for diagnosis of the metabolic syndrome such as the National Cholesterol Education Programme Adult Treatment Panel III (Expert Panel on Detection, 2001), World Health Organization (W.H.O., 1999), The European Group for the Study of Insulin Resistance (Balkau *et al.*, 2002), and the American Association of Clinical Endocrinologist (Bloomgarden, 2003). A simple measurement

for the metabolic syndrome also known as “hypertriglyceridaemic waist” depends on waist circumference >90.0cm and blood triglyceride concentration greater than 2.0 mmol/L (Lemieux *et al.,* 2000). The World Health Organization (WHO) consultation group in 1998 outlined a provisional working definition for the metabolic syndrome which was revised in in 1999. Metabolic syndrome was defined as insulin resistance with two or more of hypertension – blood pressure ≥140/90 mmHg and/or on antihypertensive medication; central obesity(waist-to-hip ratio >0.90 in males or >0.85 in females and/or BMI >30 kg/m2) ; dyslipidaemia (triglyceride ≥1.7 mmol/L and/or HDL-c <0.9 mmol/L in males and <1.0 mmol/L in females); Microalbuminuria (urinary albumin excretion rate >20 µg/min or an albumin: creatinine ratio >20 mg/g). Insulin resistance was defined as type 2 diabetes, impaired fasting glucose (IFG), impaired glucose tolerance (IGT), or for those with normal fasting glucose (<6.1 mmol/L) a glucose uptake below the lowest quartile for population under hyperinsulinaemic euglycaemic conditions. In addition to insulin resistance, higher blood pressure and BMI are sufficient for a diagnosis of metabolic syndrome according to ATP III criteria. The special testing of glucose status beyond routine clinical assessment may be necessary to diagnose metabolic syndrome is a potential disadvantage of the WHO criteria (Ref). Afterward, new working definition for the metabolic syndrome was developed by the National Cholesterol Education Programme Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in adults (NCEP-ATPIII, 2001). Metabolic syndrome was defined by three or more of the following: Hypertension (blood pressure ≥130/85 mmHg and/or on antihypertensive medication); Hypertriglyceridemia (triglyceride ≥1.70 mmol/L); Low HDL cholesterol <1.0 mmol/L in males and <1.3mmol/L in females; fasting plasma

glucose ≥6.1mmol/L; central obesity (waist circumference >102 cm in males and >88 cm in females).

There are multiple marginal risk factors that can impart on the high risk for CVD. Most individual who meets the ATP III criteria will be insulin resistant though explicit demonstration of insulin resistance is not required for diagnosis. The presence of type 2 diabetes doesn't exclusively diagnose metabolic syndrome (Ref).

A person is said to have metabolic syndrome if one has insulin resistance or hyperinsulinaemia for only non-diabetic subjects and two or more of the following: Dyslipidaemia (triglyceride >2.0 mmol/L and/or HDL cholesterol <1.0 mmol/L or treated for dyslipidaemia) ; hypertension (blood pressure >140/90mmHg and/or on antihypertensive medication); fasting plasma glucose >6.1 mmol/L; waist circumference >94 cm in males and >80 cm in females for central obesity index according to the European Group for the Study of Insulin Resistance (Balkau *et al.*, 2002). NCEP ATPIII and WHO criteria for metabolic syndrome make up the definition for European Group for the Study of Insulin Resistance criteria for the metabolic syndrome.

The American Association of Clinical Endocrinologist (AACE) proposed a third set of criteria for the insulin resistance syndrome which appears to be a hybrid of those of NCEP ATP III and WHO metabolic syndrome. BMI ≥25kg/m^2 for overweight/ obesity, elevated triglyceride >1.69 mmol/L, low HDL cholesterol (males <1.04 mmol/L and females <1.29 mmol/L) and hypertension ≥130/85mmHg were the main risk factor components and their cutpoints for abnormality (Ref).

2.12 GLYCATED HAEMOGLOBIN AND DIABETES

Epidemiological studies have confirmed that hyperglycaemia is the most important factor in onset and progress of diabetes complications, both in type 1 and type 2 diabetes

mellitus. Mechanisms connecting hyperglycaemia with long term complications of diabetes have been investigated. Among others, a large number of useful proofs indicated the involvement of nonenzymatic glycation processes (Lyons & Jenkins, 1997). Nonenzymatic glycation is the process by which glucose is chemically bound to amino groups of amino acids of proteins, without the involvement of enzymes. It occurs by a series of chemical reactions described by a chemist Maillard (1912). Maillard reactions are complex and multilayer and can be analyzed in three degrees. The first reaction is a classical covalent reaction in which, by means of N-glycoside bonding, a sugar-protein complex is formed. (Amadori rearrangement). It is an early product of nonenzymatic glycation, an intermediate which is a precursor of all later compounds. The second degree includes the formation of numerous intermediary products among which some are very reactive and further continue with glycation reactions. The third, final phase, consists of a complex polymerization reaction of the second stage products, in the process of which heterogeneous structures called advanced glycation endproducts (AGEs) are formed (Vlassara *et al.*, 1994; Singh *et al.*, 2001). It was believed that the primary mechanism in Maillard reactions was exclusively the pathway that originated from high glucose concentration. However, recent data show that, in spite of the fact that sugars are the main precursors of AGE compounds, numerous intermediary metabolites, i.e. αoxoaldehydes also creatively participate in nonenzymatic glycation reactions. Such intermediary products are generated during glycolysis (methylglyoxal) or lipid peroxidation (Lyons *et al.*, 1997) and they can also be formed by autooxidation of carbohydrates (glyoxal). Another route is the polyolic pathway by which glucose is metabolized through sorbitol, then fructose to α-oxoaldehydes. Alpha-oxoaldehydes modify AGEs surprisingly fast, in contrast to classical Maillard reactions which are very slow. A classic example of nonenzymatic glycation is the formation of glycated

haemoglobin (GHb), also commonly referred to as glycosylated haemoglobin, glycohaemoglobin, HbA1C, HbA1, or A1C. Glycated haemoglobin is a term used to describe a series of stable minor haemoglobin components formed slowly and nonenzymatically from haemoglobin and glucose. HbA1C has been the first studied glycated protein, but it was soon discovered that other structural and regulatory proteins, are also subject to nonenzymatic glycation, forming glycation endproducts. The initial step in the reaction is the condensation of a free primary amine on haemoglobin with the carbony1 of the glucose, resulting in the formation of a Schiff base, that is, early Maillard reaction (1912). This Schiff base is not stable and may either dissociate or undergo an Amadori rearrangement to form a stable ketoamine. There is now considerable evidence for an Amadori-type rearrangement of the adduct glucose with the NH2-terminal valine of the β-chain (HbA1C) as well as the NH2terminal valine of the α-chain and for ε-amino groups of certain lysine residues on α- and β-chains. Since haemoglobin circulates in each erythrocyte for about 120 days, there is some opportunity in this cell for late Maillard reactions or nonenzymatic reactions to occur (the products of these reactions are referred to as advanced glycation end products [AGEs]), and the extent of these changes appears to correlate with GHb values (Makita *et al.*, 1992). In the formation of AGEs, the Amadori product is degraded into deoxyglucosones, which react again with free amino groups to form other products (Angyal, 1979). The rate of formation of GHb is directly proportional to the ambient glucose concentration. Glycation has both physiological and pathophysiological significance in tissues that are longer lived (connective tissue, vascular endothelium, etc.). In physiological conditions glycation can be detected in the ageing process (Vlassara *et al.*, 1994), and the reactions are significantly faster and more intensive with frequently increased glucose concentrations. In diabetology the importance of these

processes is manifest in two essential issues: (1) effect of protein glycation on changes in their structure and function and (2) Use of glycated protein levels as a parameter of integrated glycaemia (Bucala & Cerami, 1992; Brownlee, 2000). Since erythrocytes are freely permeable to glucose, the level of

GHb in a blood sample provides a glycaemic history of the previous 120 days, the average erythrocyte lifespan. GHb most accurately reflects the previous 2−3 months of glycaemic control. However, recent (i.e. 3 – 4 weeks earlier) plasma glucose levels contribute considerably more (50%) to the level of HbA1C (Tahara, 1993) than do long-past (i.e., 3 – 4 months earlier) plasma glucose levels (10%). Measurements of glycated proteins, primarily haemoglobin and serum proteins, have added a new dimension to the assessment of glycaemia. Blood and urine glucose and urine ketone tests cannot provide the patient and health care team with an objective measure of glycaemia over an extended period of time. However, with a single measurement, glycated proteins can quantify average glycaemia over weeks and months, thereby complementing day-to-day testing (Singer *et al.*, 1989)of blood and urine glucose and urine ketones. It also provides an additional advantage because GHb values are free of day-to-day glucose fluctuations and are unaffected by exercise or recent food ingestion. HbA1C is currently considered the best index of metabolic control for diabetic patients in clinical settings (Goldstein, 1984; Nathan *et al.*, 1984) and participants in epidemiological studies. Routine use of GHb testing in all patients with diabetes mellitus is recommended by the American Diabetes Association (2004), first to document the degree of glycaemic control at initial assessment, then as part of continuing care. GHb is also used as a measure of risk for the development of micro- and macrovascular diabetic complications (Moss *et al.*, 1994; Krolewski *et al.*, 1995). The test is also being used increasingly by quality assurance programmes including the American Diabetes Association to assess the quality of diabetes care (Davidson, 1998). Elevated C-reactive protein concentrations increased with

increasing HbA1C levels. This suggests an association between glycaemic control and systemic inflammation in diabetics (King *et al.,* 2003). HbA1C concentration is also related to prevalent coronary disease or carotid intimal thickening in nondiabetic individuals (Vitelli *et al.,* 1997). HbA1C has been suggested as a diagnostic and screening tool for diabetes mellitus in the general population (Rohlfing *et al.,* 2000). In acutely ill patients with random hyperglycaemia at hospital admission, an HbA1C level >6.0% reliably diagnoses diabetes mellitus, and an HbA1C level <5.2% reliably excludes it (Greci *et al.,* 2003) It has been suggested that, in diabetic patients, management plan should be adjusted to achieve normal or near normal glycaemia with an A1C goal of

<7% (Lawson *et al.,* 1999; Stratton *et al.,* 2000). More stringent goals (i.e., a normal A1C <6%) can be considered in individual patients and in pregnancy. Less stringent treatment goals may be appropriate for patients with a history of severe hypoglycaemia, patients with limited life expectancies, very young children or older adults and individuals who have disease conditions associated with diabetes mellitus.

2.13 INFLAMMATION AND DIABETES

2.13.1 Acute-phase proteins and diabetes

Inflammation also induces high systemic levels of acute-phase proteins. In acute inflammation, these proteins prove beneficial, however in chronic inflammation they can contribute to amyloidosis (Saikumar *et al.,* 1998). Proteins such as C-reactive protein, serum amyloid A, and serum amyloid P, which cause a range of systemic effects including: Loss of appetite, Fever, hypertension, reduced sweating, muscle weakness and somnolence (Kumar *et al.,* 1998).

2.13.2 Sialic acid and diabetes

Sialic acid is a generic term for the N- or O-substituted derivatives of neuraminic acid, a monosaccharide with a nine-carbon backbone (Varki, 2008). Sialic acids are mostly

found in glycoproteins and gangliosides widely distributed in animal tissues and to a lesser extent in other organisms, ranging from plants and fungi to yeasts and bacteria (W.H.O., 2003). Elevation of serum or plasma sialic acid concentration is a marker of the acute-phase response (Sabzwari *et al.*, 2006). Most acute phase glycoproteins have sialic acid as the terminal sugar on their oligosaccharide chain and thus could be used as a marker of acute phase protein and inflammation in pathogenic conditions (Pickup, 2004). In prospective studies some authors (Lindberg *et al.*, 1992; Crook *et al.*, 1993; 1994) investigated the association between sialic acid and cardiovascular disease and reports that an elevated serum concentration of sialic acid is a strong predictor in the general population of cardiovascular death.

Elevated sialic acid concentration has been found in a study among diabetes mellitus patients with complications (Laing *et al.*, 2003). However, this association remains inconsistent as reported by several authors (Browning *et al.*, 2004; Sabzwari *et al.*, 2006; Rahman *et al.*, 2009). In addition, sialic acid was observed to be involved in the pathogenesis of diabetic microangiopathic complications as elevated levels were directly proportional to the severity of microangiopathic complications (Crook *et al.*, 2001).

2.13.3 C-reactive protein

The C-reactive protein (CRP) is found in the blood plasma, the levels of which rise in response to inflammation (i.e., C-reactive protein is an acute-phase protein). Its physiological role is to bind to phosphocholine expressed on the surface of dead or dying cells (and some types of bacteria) in order to activate the complement system via the C1Q complex. (Pradhan *et al.*, 2001). CRP is synthesized by the liver in response to factors released by macrophages and fat cells (adipocytes). C-reactive protein was the first pattern recognition receptor (PRR) to be identified (Mantovani *et al.*, 2004).

Recent advances in basic science have established a fundamental role for inflammation in mediating all stages of this disease from initiation through progression and, ultimately, the thrombotic complications of atherosclerosis (Lau *et al.*, 2005). These new findings provide important links between risk factors and the mechanisms of atherogenesis. Clinical studies have shown that this emerging biology of inflammation in atherosclerosis applies directly to human patients. Elevation in markers of inflammation predicts outcomes of patients with acute coronary syndromes, independently of myocardial damage (Sabzwari *et al.*, 2006)

Additionally low-grade chronic inflammation, as indicated by levels of the inflammatory marker C-reactive protein, prospectively defines risk of atherosclerotic complications, thus adding to prognostic information provided by traditional risk factors (Hansson *et al.*, 2002). Moreover, certain treatments that reduce coronary risk also limit inflammation. In the case of lipid lowering with statins, this antiinflammatory effect does not appear to correlate with reduction in low-density lipoprotein levels. These new insights into inflammation in atherosclerosis not only increase our understanding of this disease but also have practical clinical applications in risk stratification and targeting of therapy (Libby *et al.*, 2002).

2.14 MICROALBUMIN AND DIABETES

A urine microalbumin test detects very small levels of a blood protein especially albumin in urine. A microalbumin test help in early detection of renal insufficiency in people who have a risk of kidney disease (Lewis *et al.*, 1993). Microalbumin tests are recommended chronic diseases such as type 1 diabetes, type 2 diabetes or high blood pressure since these conditions are mostly associated with kidney damage (Lea & Nicholas, 2002).

Chapter 3

MATERIALS AND METHODS

3.1 STUDY PARTICIPANTS

This case-control study recruited a total of 200 participants comprising 50 healthy individuals and 150 patients with type 2 diabetes of which 41 have developed diabetic nephropathy 27 have developed diabetic retinopathy and 82 had no complications.

Sample size justification

Using the Cochran formular, an estimated prevalence of 6.4%, standard normal variation at 95% confidence level and a marginal error of 5%. The minimum sample size was calculated to be 92. However, for the purpose of this study and based on the small sample size for the previous study, the sample size was increased to 200.

$$N = \frac{t^2 \, x \, P(1-P)}{m^2}$$

Where; N: sample size, t: standard normal variation at 95% confidence level (critical value of 1.96), P: estimated prevalence rate, m: margin of error

3.2 STUDY SITE

The study was conducted at the Diabetic Clinic of the Tema General Hospital, as well as the Eye Clinic and the Chemical Pathology units of the Tema General Hospital.

Tema General Hospital is located in the Tema Metropolitan area which has a total projected population of 403,943. It is the largest Public Health institution in the Metropolis. It was constructed in 1954 by J.W. Harrow and Sons Limited and handed over to Ghana government in 1962. Its catchment area includes the whole of Tema

Metropolis, its satellite towns and villages and extends as far as Sakumono, Lashibi and Nungua. It has twelve wards and 294 bed capacity and provides 24 hour Specialist and General service to both in-patients and out-patients.

The hospital is the main referral center within the Tema Metropolis.

3.3 STUDY POPULATION

Ghanaian diabetes, attending the diabetic out-patient's clinic of the hospital 150 consecutive diabetic patients with diabetes more than one year who met the inclusion criteria were selected for the study. as well as fifty (50) Ghanaian healthy individuals (controls) age- and sex-matched who met the inclusion criteria were selected.

3.4 PARTICIPANT RECRUITMENT

Recruitment was based on previous and current symptoms and test results as well as medication profile. Control recruitment was based on normal glucose tolerance and absence of diabetes within first-degree relatives.

3.4.1 Inclusion Criteria

Participants who have been diagnosed of diabetes mellitus and were 40 years and above and were on diet with oral hypoglycaemic drugs were included in this study. Participants apparently healthy with normal glucose tolerance whose first-degree relatives do not have diabetes mellitus, and were 40 years and above, who are without diabetic related complications were considered as controls.

3.4.2 Exclusion criteria

Diabetic participants who were non-Ghanaian, pregnant women, those with type 1 diabetes, as well as those who presented with chronic inflammation from other infections were excluded

3.5 ETHICAL CONSIDERATION

The research protocol was reviewed and approved by the Committee for Human Research, Publications and Ethics (CHRPE) of KNUST and the management of Tema General Hospital. The objectives and benefits of the study were explained to the diabetic patients, control or healthy control subjects at the time of initial data collection, and verbal and written consent were obtained from them.

3.6 DATA COLLECTION INSTRUMENT

A standard questionnaire was used to collect information on socio-demographic and patient's profile such as age, sex, tribe, duration of diabetes, presence of other metabolic and infectious diseases and family history of common metabolic diseases. Others were current and previous medication, intake of pharmacological agents, such as drugs including contraceptives, tobacco and alcohol, and specific physiological states such as pregnancy, stress and excessive exercise. An additional profile for control or healthy control subjects included presence of diabetes within first-degree relatives.

3.7 ANTHROPOMETRIC MEASUREMENTS

3.7.1 Weight and Height

With participants in lightweight clothing and without shoes, body weight and height were measured using a standard physician's scale and stadiometer. Results were reported to the nearest 0.1 kg and 0.5 cm respectively. BMI was calculated as weight/height^2 (kg/m^2).

3.7.2 Waist circumference

Waist circumference was measured with a tape measure. Participants were made to stand and during mid-respiration at the narrowest indentation midway between the lowest rib and the

iliac crest and at the level of the umbilicus measurements were taken and reported to the nearest 0.1 cm.

Formula calculating for calculating anthropometric indexes

$$\boldsymbol{BMI} = \frac{Weight\ (Kg)}{Height\ (m)^2}$$

$$\boldsymbol{BAI} = [(HC + Height) \times 1.5] - 1.8$$

$$\boldsymbol{VAI\ (Females)} = \frac{WC}{36.58 + (1.89 + BMI)} \times \frac{TG}{0.81} \times \frac{1.52}{HDL}$$

$$\boldsymbol{VAI\ (Males)} = \frac{WC}{39.68 + (1.88 + BMI)} \times \frac{TG}{1.03} \times \frac{1.31}{HDL}$$

3.8 BLOOD PRESSURE MEASUREMENTS

Trained medical personnel used mercury sphygmomanometer and auscultory methods to measured systolic and diastolic blood pressures and results were reported. The procedure was repeated after 30 min of rest, at 5 min intervals, and their mean values calculated.

3.9 DEFINITION AND CLASSIFICATION OF METABOLIC SYNDROME

Metabolic syndrome were diagnosed using the criteria recommended by the NCEP ATPIII, that is, the presence of three or more of the following risk factors:

Hypertriglyceridemia (triglyceride ≥1.70 mmol/L); Central obesity (waist circumference in males >102 cm and females >88 cm); Hypertension i.e. blood pressure ≥130/85 mmHg and/or on antihypertensive medication; Low HDL cholesterol (HDL cholesterol in males <1.00 mmol/L and in females <1.30 mmol/L); and ; Hyperglycaemia i.e. a fasting glucose ≥6.1mmol/L.

3.10 SAMPLE COLLECTION AND PROCESSING

Venous Blood samples were taken from subjects after a 8- to 12-hour overnight fast. Venous blood was collected into fluoride-oxalate bottles; fluoride inhibits the enolase reaction in glycolysis by forming magnesium fluorophosphate complex, plain vacutainer tubes (Becton Dickinson, Rutherford, N.J.) and anticoagulated (EDTA) tubes. The blood samples in the fluoride bottles were immediately centrifuged at 1,000g for 5 minutes to prevent glycolysis from occurring and analyzed for glucose levels. The samples in the vacutainer tubes were also centrifuged after 30 minutes at 1,000g for 15 minutes at room temperature. Serum was separated into plain sample containers and frozen at –20 ºC for 2 weeks to 1 month and until analyzed.

3.11 BIOCHEMICAL ASSAY 3.11.1 Determination of glucose, total cholesterol, triglyceride and HDL

cholesterol using BT 3000 Chemistry Auto Analyzer and its reagent kits

Glucose, total cholesterol, triglycerides and HDL cholesterol were measured using enzymatic methods and the procedures outlined in the operator's manual and BT 3000 Chemistry auto analyzer. The procedures are described by the manufacturer for glucose, cholesterol triglyceride and HDL cholesterol (BT 3000 Chemistry auto analyzer Operator's manual). Basically, working reagents were prepared according to manufacturer's instructions. Programming, calibration (after loading a new reagent) using provided standards, and running of normal and abnormal controls to ensure that the controls lie within acceptable ranges were done. The instrument automatically compared calibration factors against preprogrammed acceptance criteria. Assay of samples were then carried out as directed in the operator's manual. The standards were calibrated against international W.H.O approved reference material NIBSC

66/304. LDL cholesterol was derived from Friedewald's formula [LDL cholesterol (mmol/L) = total cholesterol (mmol/L) – HDL cholesterol (mmol/L) − triglyceride (mmol/L)/2.2] using the auto analyzer (Friedewald *et al.*, 1972).

3.11.2 Sialic acid assay

The double-antibody sandwich enzyme-linked immunosorbent one-step process assay (ELISA) was used to assay the level of Lipid –bound sialic acid (LSA) in the samples. The standard, test sample and HRP-labeled Lipid –bound sialic acid (LSA) antibodies, were added to enzyme wells which are Pre-coated with Lipid –bound sialic acid (LSA) antibody. The wells are then incubated at 37 ˚C for 60 minutes and washed for 5 times to remove the uncombined enzyme. Upon adding the Chromogen Solution A and B, the color of the liquid changes into blue, and the reaction with the acid causes the color to become yellow. The depth of the concentration of the Lipid – bound sialic acid (LSA) measured at 450nm wavelength positively correlates with that of the samples.

3.11.3 Glycosylated hemoglobin (HbA1c)

The A_1 fast fraction – cation exchange method was used to estimate the level of glycated hemoglobin of the participants.

Principle

A haemolysed preparation of whole blood was mixed continuously for 5 minutes with a weak binding cation -exchange resin. During this time, HbA binds to the resin. The non-glycosylated haemoglobin binds to the resin leaving GHb free in the supernatant containing the glycosylated haemoglobin. After the mixing period, a filter was used to separate the supernatant containing the glycosylated haemoglobin from the resin. The

GHb percentage was determined by measuring the absorbance at 415 nm of the GHb fraction and the total Hb fraction. The ratio of the two absorbances gives the percentage of glycosylated haemoglobin (GHb).

The percent HbA1C in the sample is then calculated as follows:

$$\%\ \mathrm{HbA_{1C}} = \frac{[\mathrm{HbA_{1C}}]}{[\text{Total Haemoglobin}]} X\ 100$$

3.11.4 Ozotex-C-reactive protein (Latex Agglutination Method)

Principle

Uniform latex agglutination particles are coated with Anti-Human CRP. The specimen containing CRP on mixing with latex reagent agglutinates showing a positive result. If CRP is absent, there will be no agglutination, indicating a negative test results.

Procedure

Whole blood drawn into a serum separator tube is centrifuged after it has clotted to obtain a fresh serum. The serum was serially diluted as 1: 2, 1:4, 1: 8, 1:16, 1:32, and 1: 64 using normal saline. One drop each of diluted serum sample using sample dropper is placed in each circle of the glass slide. The content of each slide is mixed separately and spread in the entire circle with the mixing sticks provided in the kit. Rocking of the slide is done gently for two minutes. Agglutination within two minutes is a positive test and indicates presence of CRP in the test specimen. No agglutination up to 2 minutes is a negative test and indicates absence of CRP in test specimen. The highest dilution that shows clear cut agglutination within 2 minutes indicate CRP titre, the approximate CRP concentration can be obtain by multiplying titre by the

sensitivity of the test.

$$\text{CRP in mg/dl} = \frac{\text{Highest dilution showing clear agglutination (D)}}{\text{Sensitivity of the test (S)}}$$

Where S= 0.6mg/dl.

3.12 STATISTICAL ANALYSIS

Results were expressed as mean ± S.D. except where otherwise stated. Statistical analysis was performed using SPSS version 20.0 (SPSS Inc.) and GraphPad prism 5 for Windows. Normal distribution and homogeneity of the variances were tested using Kolmogorov-Smirnov and Levène tests, respectively. Student t-test was used to compare the significance of the difference in the mean values of any two groups and chi-square analysis was used to compare frequency between the two groups. Linear regression analysis was used to study the association between the parameters. Correlations between parameters were analyzed using the Pearson R test for variables with normal distribution and the Spearman test for variables with non-normal distribution. $P<0.05$ was considered statistically significant.

Chapter 4

RESULTS 4.1 BASELINE CHARACTERISTICS AND MEASURES OF ANTHROPOMETRY OF THE PARTICIPANT

Table 4.1 presents the baseline characteristics of the participants. The mean ages of the diabetic patients and the non-diabetics were 58.90 ± 12.43 and 54.88 ± 17.90 years respectively. Furthermore, majority of the diabetic patients, 28% (42), were between the ages of 60-69 years and 32.0% of the non-diabetics were aged 50-59 years. Assessments of obesity using Waist circumference was significantly (P<0.0001) higher in the diabetics than the non-diabetics (Table 4.1). Waist-to-hip ratio and BMI comparisons showed no mean significant differences, however, overweight (35.3%) and obesity (36.0%) was more prevalent in the diabetics than in the non-diabetics.

Table 4.1 Demographic and anthropometric characteristics of study participants

Variable	**Subjects** (n = 150)	**Controls** (n = 50)	**P-value**
Age (Mean ± SD)	58.90 ± 12.43	54.88 ± 17.90	0.080
Gender n (%)			**< 0.0001**
Male	58 (38.7)	36 (72.0)	
Female	92 (61.3)	14 928.0)	
Age group n (%)			0.063
<30	3 (2.0)	5 (10.0)	
30-39	8 (5.3)	7 (14.0)	
40-49	25 (16.7)	8 (16.0)	
50-59	38 (25.3)	16 (32.0)	
60-69	44 (29.3)	7 (14.0)	
70-79	27 (18.0)	4 (8.0)	
≥ 80	5 (3.3)	3 (6.0)	
Marital status n (%)			**0.001**

Single	27 (18.0)	18 (36.0)	
Married	89 (59.3)	32 (64.0)	
Divorced	7 (4.7)	0 (0.0)	
Widowed	27 (18.0)	0 (0.0)	
Occupation n (%)			**0.022**
None	50 (33.3)	7 (14.0)	
Informal	72 (48.0)	28 (56.0)	
Formal	28 (18.7)	15 (30.0)	
Educational status n (%)			0.078
None	21 (14.5)	15 (30.0)	
Basic	60 (41.4)	17 (34.0)	
Secondary	42 (29.0)	14 (28.0)	
Tertiary	22 (15.2)	4 (8.0)	
WC (cm)	92.44 ± 13.27	79.80 ± 11.14	**< 0.0001**
WHR	0.90 ± 0.07	0.89 ± 0.17	0.451
BMI n (Kg/m2)	28.55 ± 5.75	28.69 ± 5.10	0.880
BMI n (%)			0.490
Underweight	3 (2.0)	0 (0.0)	
Normal	40 (26.7)	10 (20.0)	
Overweight	53 (35.3)	22 (44.0)	
Obese	54 (36.0)	18 (36.0)	

Values are Mean ±SD, Differences is significant at P<0.05, Percentages are denoted in brackets. WC = Waist Circumference, WHR = Waist to Hop ratio, BMI = Body Mass Index,

4.2 BIOCHEMICAL CHARACTERISTICS, AND INDICES ADIPOSITY OF STUDY PARTICIPANTS.

Blood glucose, glycated hemoglobin, adiposity indices and blood pressure (SBP/DBP) were significantly (P< 0.05) increased in the patients with diabetes compared to the non-diabetics (Table 4.2). Serum inflammatory markers were also significantly different with elevated sialic acid (P<0.0001) and C-reactive protein (p=0.026) in the diabetics. However, serum lipid profile although increased in the diabetics, showed no statistically

significant difference between diabetics and nondiabetics as TC, TG and HDL were compared (P > 0.05) except for LDL (P= 0.03) (Table 4.2).

Table 4.2 Glycemic indices, inflammatory parameters, Adiposity indices and lipid profile among the T2DM patients and the non-diabetics

Variable	Cases (n = 150)	Controls (n = 50)	P-value
FBG (mmol/l)	9.36 ± 3.83	4.78 ± 0.63	< **0.0001**
HBA1c (%)	7.46 ± 1.38	6.69 ± 1.24	**0.001**
Adiposity indices			
VAI	1.72 ± 0.20	1.00 ± 0.09	**0.039**
BAI	32.60 ± 7.91	23.26 ± 6.13	**<0.0001**
Blood Pressure (mmHg) SBP			
	128.10 ± 17.08	120.0 ± 7.28	**0.001**
DBP	81.67 ± 8.43	78.60 ± 7.29	**0.022**
Inflammatory parameters			
SSA (mg/dL)	196.67 ± 87.21	122.49 ± 30.38	**<0.0001**
CRP	0.12 ± 0.03	0.00 ± 0.00	**0.026**
Lipid profile TC (mmol/L)	5.10 ± 1.26	4.77 ± 1.17	0.098
TG (mmol/L)	1.20 ± 0.49	1.16 ± 0.80	0.681
HDL-CHL (mmol/L)	1.38 ± 0.40	1.50 ± 0.64	0.121
LDL-CHL (mmol/L)	3.56 ± 1.30	3.11 ± 1.08	**0.030**

FBG=Fasting Blood Glucose, HB=Haemoglobin, HBA1C=Glycated Haemoglobin, VAI=Visceral Adiposity Index, BAI=Body Adiposity Index, SBP= Systolic Blood Pressure, DBP=Diastolic Blood Pressure, SSA=Serum Sialic Acid, CRP= C - reactive protein, TC=Total Cholesterol, TG=Triglycerides, HDL=High Density Lipoprotein, LDL=Low Density Lipoprotein

4.3 AGE DISTRIBUTION, MEASURE OF ANTHROPOMETRY, AND PREVALENCE OF DISEASE COMPLICATION

The mean ages of the diabetic male and female patients were 59.17 ± 13.72 and 58.73 ± 11.63 respectively. Waist circumference and waist-to-hip ratio were not significantly different (*P*> 0.05) in the males compared to the females (Table 4.3). Measure of adiposity indicated increase in visceral adiposity index (VAI) and body adiposity index (BAI) in the female with BAI difference being statistically significant (P= 0.001). BMI comparison showed significant differences, with overweight (62.3%) and obesity

(74.1%) more prevalent in the females than the male diabetics. Diabetic nephropathy and retinopathy were also more prevalent in the female diabetics (68.3%, 66.7% respectively) than the male diabetics (31.7%, 33.3% respectively) (Table 4.3).

Table 4. 3 Demographic clinical anthropometry and adiposity indices of participants stratified by gender.

Variable	Male (n = 58)	Female (n = 92)	P-value
Age (Mean ± SD)	59.17 ± 13.72	58.73 ± 11.63	0.823
Age group n (%)			0.197
<30	3 (100.0)	0 (0.0)	
30-39	2 (25.0)	6 (75.0)	
40-49	8 (32.0)	17 (68.0)	
50-59	11 (28.9)	27 (71.1)	
60-69	20 (45.5)	24 (54.5)	
70-79	12 (44.4)	15 (55.6)	
≥ 80	2 (40.0)	3 (60.0)	
WC (cm)	91.59 ± 12.77	92.97 ± 13.62	0.539
WHR	0.90 ± 0.08	0.91 ± 0.07	0.611
Adiposity indices			
VAI	1.35 ± 0.09	1.96 ± 0.32	0.132
BAI	29.89 ± 7.34	34.31 ± 7.82	**0.001**
BMI n (Kg/m2)	26.98 ± 5.20	29.54 ± 5.88	**0.007**
BMI n (%)			**0.021**
Underweight	1 (33.3)	2 (66.7)	
Normal	23 (57.5)	17 (42.5)	
Overweight	20 (37.7)	33 (62.3)	
Obese	14 (25.9)	40 (74.1)	
Disease complication n (%)			0.348
None	36 (43.9)	46 (56.1)	
Nephropathy	13 (31.7)	28 (68.3)	
Retinopathy	9 (33.3)	18 (66.7)	

4.4 GLYCEMIC INDICES, INFLAMMATORY MARKERS AND LIPID PROFILE AMONG THE DIABETIC PATIENTS

Blood glucose, glycated hemoglobin and blood pressure (SBP/DBP) were not significantly ($P>0.05$) different in the male and female diabetics (Table 4.4). Serum

inflammatory markers also showed no statistically significant difference with increased levels of serum sialic acid and C-reactive protein in the female diabetic patients. However, serum lipid profile although increased in the female diabetics, showed no statistically significant difference between the genders as TC, TG and LDL were compared ($P > 0.05$) except for HDL ($P=0.018$) (Table 4.4).

Table 4.4 Glycemic indices, inflammatory markers, and lipid profile among the T2DM patients stratified by gender

Variable	Male (n = 58)	Female (n = 92)	P-value
FBG (mmol/l)	9.39 ± 4.45	9.33 ± 3.40	0.929
HBA1c (%)	7.21 ± 1.32	7.61 ± 1.40	0.078
Blood Pressure (mmHg)			
SBP	127.50 ± 16.99	128.48 ± 17.22	0.734
DBP	81.57 ± 8.67	81.74 ± 8.33	0.905
Inflammatory parameters			
SSA (mg/dL)	192.00 ± 96.81	199.62 ± 80.99	0.604
CRP	0.09 ± 0.04	0.13 ± 0.04	0.545
Lipid profile TC (mmol/L)	4.93 ± 1.20	5.21 ± 1.30	0.191
TG (mmol/L)	1.23 ± 0.65	1.17 ± 0.37	0.479
HDL-CHL (mmol/L)	1.28 ± 0.34	1.44 ± 0.43	**0.018**
LDL-CHL (mmol/L)	3.48 ± 1.26	3.61 ± 1.33	0.558

4.5 PROPORTION OF GLYCEMIC CONTROL AND DISEASE COMPLICATIONS

In the patients with diabetes, 14.7% were able to excellently control their blood glucose with 28.0% poorly controlling their glycaemia (Figure 4.1).

Across the various age groups as shown in fig 4.2, diabetic nephropathy (29.3%) was more prevalent in the diabetic patients aged between 70-79 years and retinopathy (44.3%) more prevalent in the patients aged 60-69 years. The patients aged < 30 years

did not present with diabetic nephropathy, however 3.7 % of them presented with diabetic retinopathy (Figure 4.2).

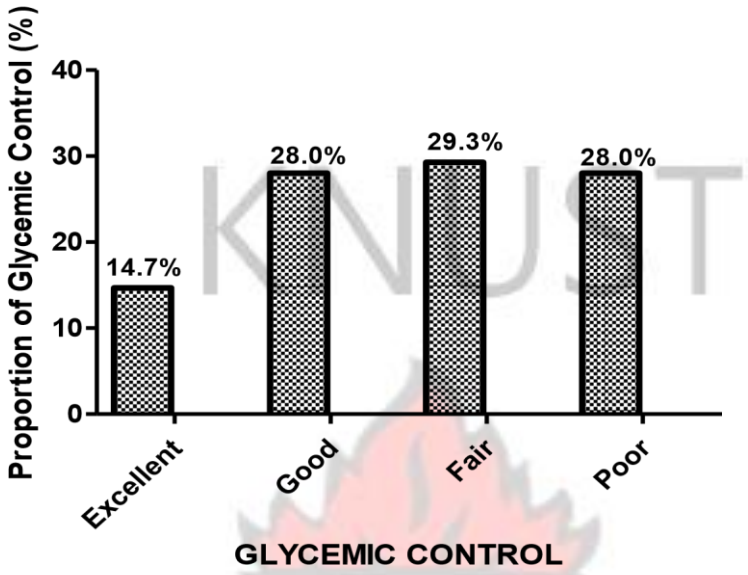


Figure 4. 1 Proportion of glycemic control of patients with T2DM assessed by HbA1c (%). Percentage is out of whole patients (n = 150).

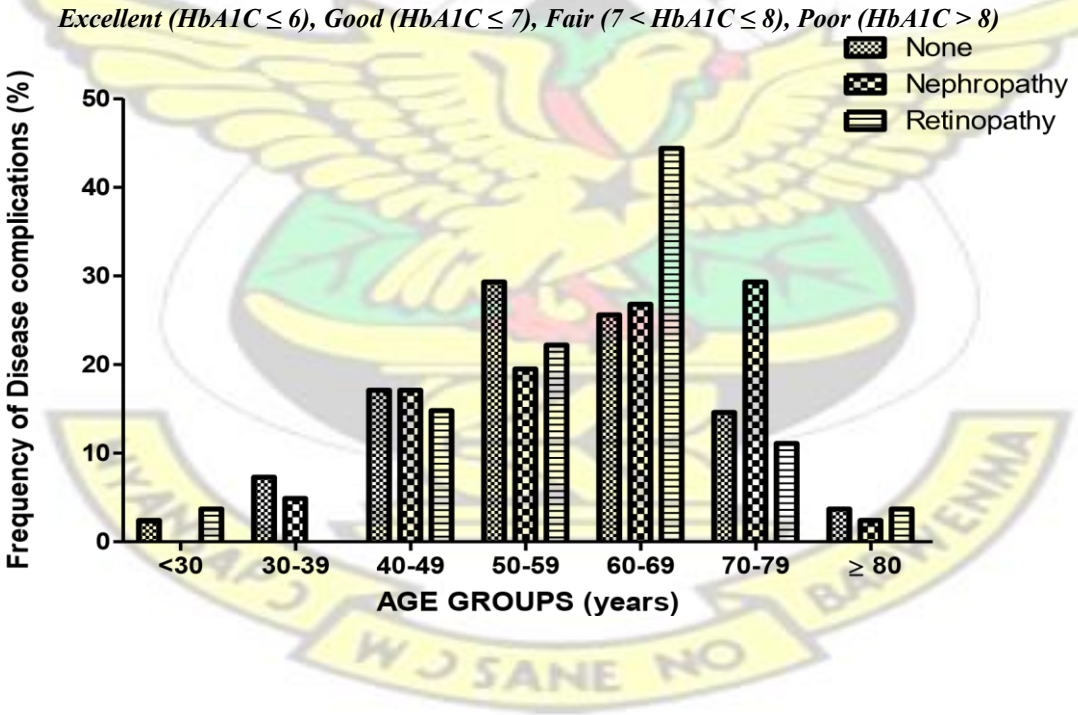


Figure 4. 2 Complications of diabetes across the various age distributions. Percentage is within the complication groups.

4.6 GLYCEMIC INDICES AND PROPORTION OF GLYCEMIC CONTROL OF PATIENTS WITH DIABETES STRATIFIED BY THE DISEASE COMPLICATION

In the patients with diabetic nephropathy, HbA1c was significantly (P< 0.05) lower compared to the diabetics with no complications and those with retinopathy (Table 4.5). ANOVA multiple comparisons of the various complications showed no significant differences in levels of serum sialic acid and C-reactive protein (P=0.065, P =0.197 respectively). Blood pressure showed significant difference across the various groups. Patients with retinopathy had significantly lower blood pressures (SBP/DBP) compared to those with nephropathy (Table 4.5). In the patients with nephropathy, 17.1% (7) excellently controlled their blood glucose whilst 14.6% (6) poorly did. On the other hand, 14.8 (4) of the patients with retinopathy excellently controlled their blood glucose whist 40.7% (11) did poorly (Table 4.5).

Table 4.5 Glycemic indices, inflammatory markers, and lipid profile among the T2DM patients stratified by disease complications Variables Disease Complication

P-value				
	None (n = 82)	**Nephropathy** (n = 41)	**Retinopathy** (n = 27)	
Age (Mean ± SD)	57.22 ± 13.06	61.46 ± 11.53	60.11 ± 11.40	0.175
FBG (mmol/l)	9.25 ± 3.57	9.39 ± 4.12	9.62 ± 4.24	0.909
HBA1c (%)	7.60 ± 1.34	6.90 ± 0.97*	7.89 ± 1.78+	**0.005**
HbA1C n (%)				0.055
Excellent	11 (13.4)	7 (17.1)	4 (14.8)	
Good	17 (20.7)	18 (43.9)	7 (25.9)	
Fair	29 (35.4)	10 (24.4)	5 (18.5)	
Poor	25 (30.50	6 (14.6)	11 (40.7)	
Blood Pressure (mmHg)				
SBP	126.89 ± 14.37	133.90 ± 23.01*	122.96 ± 11.37+	**0.022**
DBP	83.11 ± 6.22	81.22 ± 11.22	78.00 ± 8.52*	**0.021**
Inflammatory parameters SSA (mg/dL)	185.55 ± 75.69	196.53 ± 61.19	230.68 ± 135.60*	0.065

CRP	0.16 ± 0.05	0.09 ± 0.03	0.02 ± 0.02	0.197

significantly different on comparison to None group, [+] significantly different on comparison to Nephropathy group at P < 0.05, Excellent (HbA1C ≤ 6), Good (6 < HbA1C ≤ 7), Fair (7 < HbA1C ≤ 8), Poor (HbA1C > 8). DBP: Diastolic blood pressure; SBP: Systolic blood pressure; FBG: Fasting blood glucose; HBA1c: glycated haemoglobin; SSA: Sialic acid; CRP: C-reactive proteins.

4.7 OBESITY, DYSLIPIDEMIA AND RENAL FUNCTION OF THE DIABETIC PATIENTS WITH AND WITHOUT DISEASE COMPLICATIONS

Waist circumference, waist-to-hip ratio, adiposity indices and BMI showed no statistically significant difference on comparison across the disease complication (Table 4.6). Overweight was observed in 48.8% and 40.7% of the patients with nephropathy and retinopathy respectively. On the other hand, obesity was 26.8% and 33.3% in the same groups respectively. Serum lipid profile although increased in the patients with retinopathy, showed no statistically significant difference between the groups as TC, TG, LDL and HDL were compared (P > 0.05) (Table 4.6).

Table 4.6 Measure of Adiposity, and lipid profile in relation to disease complication among the T2DM patients

Variables	Disease Complication			P-value
	None	Nephropathy	Retinopathy	
	(n = 82)	(n = 41)	(n = 27)	
WC (cm)	92.56 ± 15.46	90.59 ± 10.34	94.87 ± 9.42	0.428
WHR	0.90 ± 0.07	0.89 ± 0.08	0.92 ± 0.05	0.311
Adiposity indices				
VAI	1.52 ± 0.81	2.27 ± 0.48	1.53 ± 0.75	0.251
BAI	32.64 ± 9.77	32.01 ± 5.20	33.37 ± 4.30	0.788
BMI n (Kg/m2)	28.90 ± 6.33	28.14 ± 4.89	28.09 ± 5.02	0.708
BMI n (%)				0.280
Underweight	2 (2.4)	0 (0.0)	1 (3.7)	
Normal	24 (29.3)	10 (24.4)	6 (22.2)	
Overweight	22 (26.8)	20 (48.8)	11 (40.7)	
Obese	34 (41.5)	11 (26.8)	9 (33.3)	

Lipid profile TC (mmol/L)	5.05 ± 1.19	5.07 ± 1.46	5.31 ± 1.17	0.642
TG (mmol/L)	1.22 ± 0.57	1.15 ± 0.36	1.20 ± 0.42	0.799
HDL-CHL (mmol/L)	1.38 ± 0.41	1.28 ± 0.40	1.50 ± 0.35^{+}	0.076
LDL-CHL (mmol/L)	3.47 ± 1.26	3.75 ± 1.46	3.52 ± 1.16	0.527

WC: waist circumference; WHR: Waist to hip ratio; VAI: Visceral adiposity index; BAI: body adiposity index; BMI: Body mass index; TC: Total cholesterol: TG: Triglyceride: HDL-CHL: High density lipoprotein; LDL-CHL: Low density lipoprotein.

4.8 GLYCEMIC CONTROL AMONG T2DM PATIENTS

Measures of adiposity and obesity showed no statistically significant difference when compared between patients that controlled their blood glucose well and those that did poorly, as presented in table 4.7 below. In the patients with poor glycemic control, 40.7% were obese and 29.1% overweight. Among those with good control, overweight was more prevalent (43.8%) followed by obesity (29.7%).

Levels Serum sialic acid was higher in the patients with good control than those with poor control. However, C-reactive was higher in patients with poor control and lower in those with good control. The inflammatory markers showed no significant difference on comparison (Table 4.7).

Serum lipid profile showed no statistically significant difference between the groups as TC, TG, LDL and HDL were compared ($P > 0.05$) (Table 4.7).

Table 4.7 Glycemic control among T2DM patients in relation to Measure of Adiposity, level of inflammatory parameters and lipi.ds

Variable	Glycemic Control		P-value
	Good control (*HBA1c* ≤ 7%) (n = 64)	**Poor control (*HBA1c* > 7%)** (n = 86)	
Blood Pressure (mmHg)			
SBP	128.28 ± 17.78	127.97 ± 16.65	0.911
DBP	81.41 ± 7.74	81.87 ± 8.96	0.739
Inflammatory parameters			
SSA (mgdL)	199.98 ± 82.92	194.21 ± 90.67	0.690

CRP	0.11 ± 0.28	0.12 ± 0.05	0.920
WC (cm)	92.00 ± 11.30	92.76 ± 14.63	0.729
WHR	0.89 ± 0.07	0.91 ± 0.07	0.196
Adiposity indices			
VAI	1.55 ± 0.81	1.85 ± 0.34	0.453
BAI	31.83 ± 6.93	33.17 ± 8.57	0.310
BMI n (Kg/m2)	28.55 ± 5.64	28.55 ± 5.85	0.997
BMI n (%)			0.296
Underweight	1 (1.6)	2 (2.3)	
Normal	16 (25.0)	24 (27.9)	
Overweight	28 (43.8)	25 (29.1)	
Obese	19 (29.7)	35 (40.7)	
Lipid profile TC (mmol/L)	5.24 ± 1.25	4.99 ± 1.27	0.215
TG (mmol/L)	1.19 ± 0.59	1.20 ± 0.42	0.899
HDL-CHL (mmol/L)	1.31 ± 0.41	1.42 ± 0.39	0.106
LDL-CHL (mmol/L)	3.68 ± 1.36	3.46 ± 1.24	0.308

WC: waist circumference; WHR: Waist to hip ratio; VAI: Visceral adiposity index; BAI: body adiposity index; BMI: Body mass index; TC: Total cholesterol: TG: Triglyceride: HDL-CHL: High density lipoprotein; LDL-CHL: Low density lipoprotein. DBP: Diastolic blood pressure; SBP: Systolic blood pressure; FBG: Fasting blood glucose; HBA1c: glycated haemoglobin; SSA: Sialic acid; CRP: C-reactive proteins.

4.9 RELATIONSHIP BETWEEN SERUM SIALIC ACID, BLOOD GLUCOSE AND HBA1C

In figure 4.3 below, no statistically significant relationship (P>0.05) was established between the levels of serum sialic acid and blood glucose and HbA1c among patients with diabetes (A) and those without diabetes (B). The relationship between serum sialic acid and blood glucose was not directly related, however that of HbA1c was directly related in both the diabetics and non-diabetics.

A1 **A2** **r= -0.035, *P*= 0.811, n= 50**

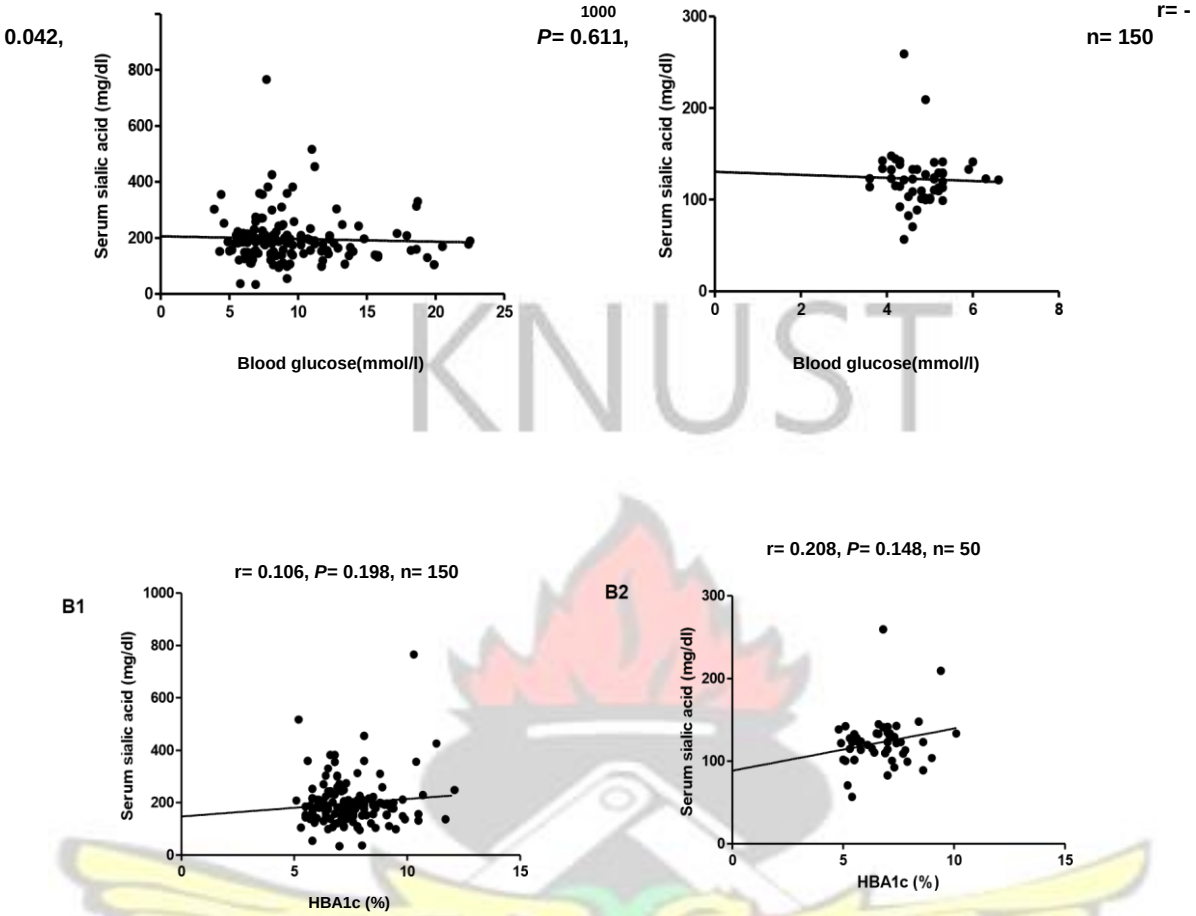


Figure 4.3 Relationship between Serum sialic acid levels, Blood glucose and HbA1c in patients with diabetes (A1, B1) and in patients without diabetes (A2, B2), r= Pearson's correlation coefficient, n = Number of patients

4.10 RELATIONSHIP BETWEEN C-REACTIVE PROTEIN, BLOOD GLUCOSE AND HbA1c

Figure 4.4 shows the relationship between C-reactive protein, blood glucose and HbA1c. C-reactive was inversely associated with blood glucose though not statistically significant (r= -0.062, P=0.454), whereas it was directly associated with HbA1c (r= 0.091, P= 0.270) (Figure 4.4).

r= -0.062, *P*= 0.454, n= 150

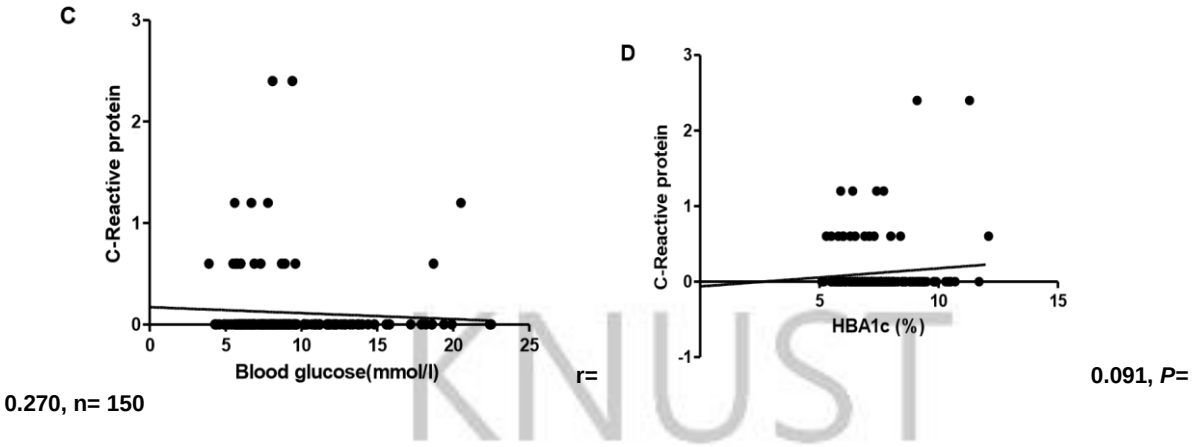


Figure 4.4 Relationship between C-reactive protein levels, Blood glucose(C) and HbA1c (D) in T2DM patients, r= Pearson's correlation coefficient, n = Number of patients

4.11 RELATIONSHIP BETWEEN THE INFLAMMATORY MARKERS, BLOOD PRESSURE AND MEASURES OF ADIPOSITY.

Inverse relationships were observed between serum sialic acid levels, blood pressure, BMI and VAI. On the other hand, C-reactive protein showed significant positive association with BMI (r= 0.341, P<0.0001) and BAI (r= 0.225, P=0.006). The markers both showed indirect association with Age and VAI (Table 4.8).

Table 4. 8 Relationship between inflammatory markers and Anthropometric variables in T2DM patients (Person's correlation)

Parameters		SSA	CRP	AGE	SBP	DBP	WHR	BMI	VAI	BAI
SSA	r	1	0.104	-0.065	-0.043	-0.103	0.105	-0.043	-0.028	0.105
	P-value		0.204	0.427	0.604	0.209	0.200	0.603	0.732	0.200
CRP	r		1	-0.035	0.023	0.119	0.079	0.341**	-0.003	.225**
	P-value			0.674	0.784	0.146	0.337	0.000	0.974	0.006
AGE	r			1	0.040	-0.07	0.060	-0.099	0.144	0.091
	P-value				0.631	0.395	0.463	0.230	0.079	0.267
SBP	r				1	0.483**	0.114	0.064	0.050	0.012
	P-value					0	0.164	0.434	0.543	0.882
DBP	r					1	0.076	0.107	0.120	0.053

	P-value	0.357	0.195	0.145	0.519
WHR	r	1	0.134	0.073	-0.033
	P-value		0.103	0.378	0.692
BMI	r		1	-0.025	.517**
	P-value			0.762	0.000
VAI	r			1	0.072
	P-value				0.383
BAI	r				1
	P-value				

r=Correlation coefficient, ** Correlation is significant at the 0.01 level (2-tailed), * Correlation is significant at the 0.05 level (2-tailed).

4.12 RELATIONSHIP BETWEEN THE INFLAMMATORY MARKERS AND LIPID PROFILE

Serum sialic acid and C-reactive protein both showed inverse relationship with total cholesterol (TC) levels, TG and LDL. However, a direct relationship between serum sialic acid and HDL with no significance (r= 0.018, P=0.825) was observed (Table 4.9)

Table 4.9 Relationship between inflammatory markers and lipids in T2DM patients (Person's correlation)

Parameters		SSA	CRP	TC	TG	HDL	LDL
SSA	r	1	0.104	-0.124	-0.149	0.018	-0.137
	P-value		0.204	0.130	0.069	0.825	0.094
CRP	r		1	-0.099	-0.032	-0.106	-0.119
	P-value			0.227	0.701	0.198	0.146
TC	r			1	0.11	0.212**	0.796**
	P-value				0.179	0.009	0.000
TG	r				1	-0.243**	0.105
	P-value					0.003	0.202
HDL	r					1	-0.075

	P-value	0.362
LDL	r	1
	P-value	

r=Correlation coefficient, ** Correlation is significant at the 0.01 level (2-tailed), * Correlation is significant at the 0.05 level (2-tailed).

4.13 LOGISTIC REGRESSION OF DETERMINANTS FOR POOR GLYCEMIC CONTROL ADJUSTED FOR AGE

The risk of having a poor glycemic control assessed by HbA1c was higher (OR= 1.63, P=0.151) for females with diabetes. Age association was observed with the age group 60-69 years at most risk (OR= 3.50, P=0.322) of having poor glycemic control. Diabetic patients with complications showed low risk of poor control assessed by HbA1c but significant in those with nephropathy (OR= 0.33, P= 0.005). Poor glycemic control determined by HbA1c was highly associated with Obesity

(OR=1.23, P= 0.633) (Table 4.10).

Table 4. 10 Logistic regression of determinants for poor glycemic control assessed by HbA1c

Variable	OR (95% CI)	P-value
Gender Male*		
	Reference	
Female	1.63 (0.84-3.17)	0.151
Age group n (%)		
<30*	Reference	
30-39	3.33 (0.20-54.53)	0.398
40-49	2.17 (0.17-27.08)	0.548
50-59	3.43 (0.28-41.32)	0.332
60-69	3.50 (0.29-41.70)	0.322
70-79	1.86 (0.15-23.00)	0.630
≥ 80	1.33 (0.07-26.62)	0.851
Disease Complication		
None*	Reference	
Nephropathy	0.33 (0.15-0.72)	**0.005**
Retinopathy	0.75 (0.31-1.84)	0.536
BMI		
Underweight	1.33 (0.11-15.96)	0.820

Normal*	Reference	
Overweight	0.60 (0.26-1.37)	0.221
Obese	1.23 (0.53-2.86)	0.633

4.14 AREA UNDER THE CURVE FOR FBG, HBA1C, AND SIALIC ACID AS DIAGNOSTIC MARKERS OF MICROVASCULAR COMPLICATIONS

Figure 4.5 shows the receiver operator curve for fasting blood glucose, glycated haemoglobin, and sialic acid as predictors of retinopathy. The area under the ROC curve for FBG and HbA1c was 0.522 and 0.534 respectively suggesting that fasting plasma glucose and HbA1c are fair marker for diagnosing diabetic retinopathy. In addition, AUC for sialic was 0.582 showing that sialic acid is a good marker for diagnosing diabetic retinopathy.

AUC for FBS and HbA1c was 0.485 and 0.342 respectively suggesting that fasting plasma glucose and HbA1c are poor tests for diagnosing diabetic nephropathy.

However, AUC for sialic was 0.573 showing that sialic acid is a good test for diagnosing diabetic nephropathy (Figure 4.6).

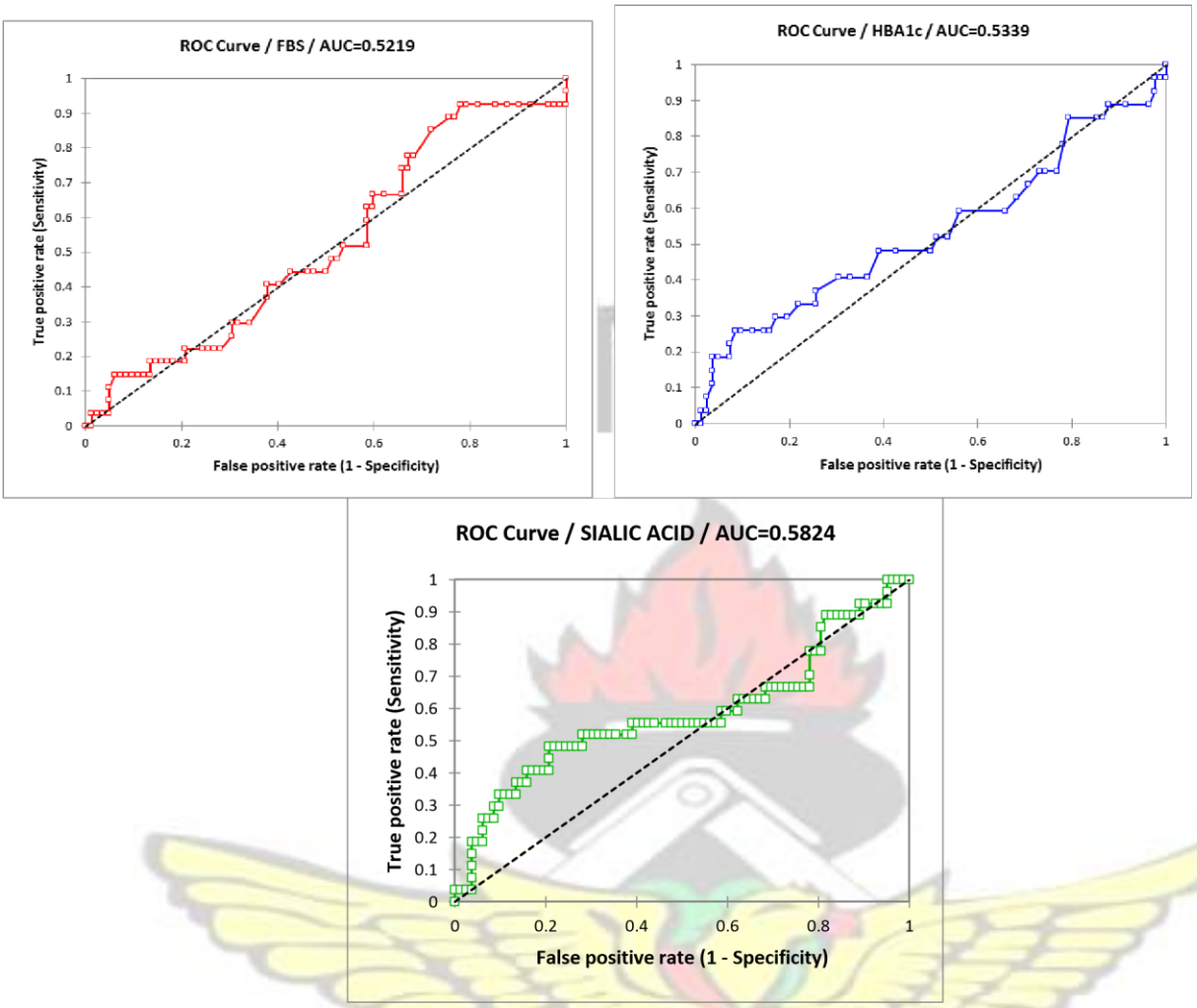
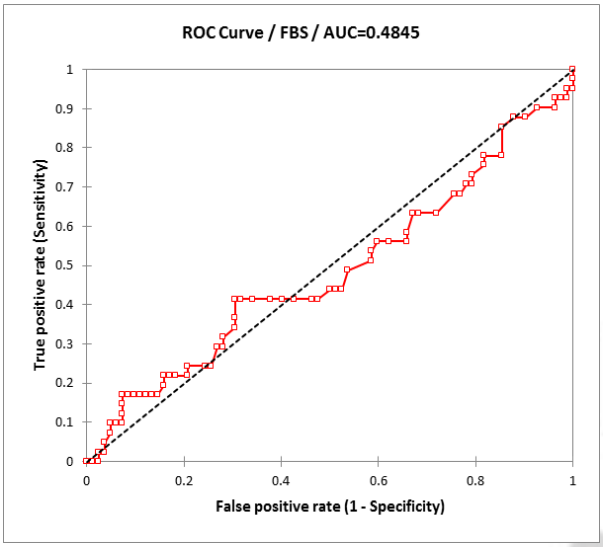
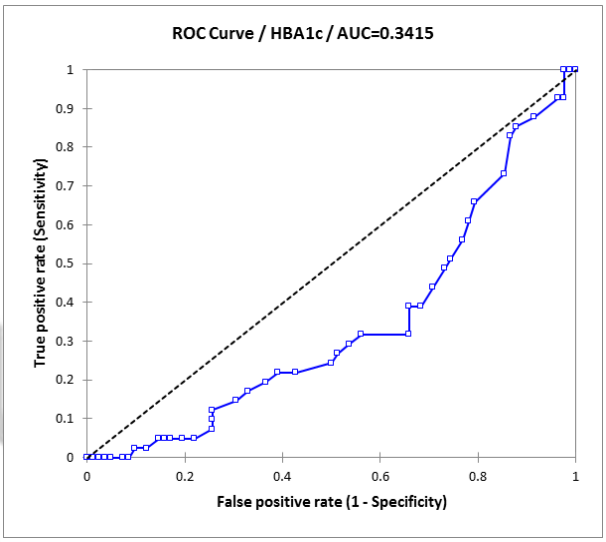


Figure 4.5: Sensitivity and specificity of biochemical markers for retinopathy





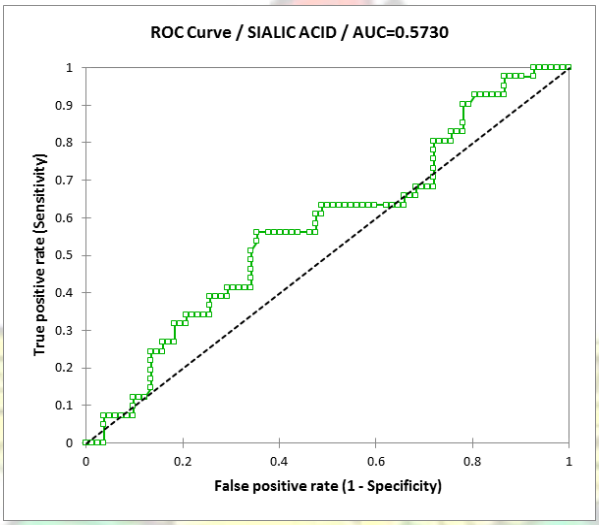


Figure 4.6: Sensitivity and specificity of biochemical markers for nephropathy

4.15 DIAGNOSTIC ACCURACY OF BIOCHEMICAL MARKERS FOR MICROVASCULAR COMPLICATIONS

The area under the ROC curve for FBG and HbA1c was 0.522 and 0.534 respectively suggesting that fasting plasma glucose and HbA1c are fair tests for diagnosing diabetic retinopathy. In addition, AUC for sialic was 0.582 showing that sialic acid is a good test for diagnosing diabetic retinopathy. AUC for FBS and HbA1c was 0.485 and 0.342 respectively suggesting that fasting plasma glucose and HbA1c are poor tests for diagnosing diabetic nephropathy. However, AUC for sialic was 0.573 showing that sialic acid is a good test for diagnosing diabetic nephropathy

Using FBS for the diagnosis of diabetic retinopathy, FBS greater than 6.5 mmol/l had a sensitivity of 92.6%, Specificity of 21.9%, Positive Predictive Value of 28.1% and a Negative Predictive Value was 90.0% (Table 4.11). For FBS greater than 9.5 mmol/l for diagnosing diabetic nephropathy, the sensitivity was also 41.5%, the specificity 69.5%, Positive predictive value 40.0% and Negative predictive value was 70.0% (Table 4.11).

In addition, diagnostic value of 9.3% for HbA1c had a sensitivity of 25.9%, Specificity of 91.4% for diagnosing diabetic retinopathy and a value of 5.3% with sensitivity of 100%, Specificity of 2.4% for diagnosing diabetic nephropathy (Table 4.11).

Sialic acid value of 205.4 had a sensitivity of 48.1%, Specificity of 79.3% for diagnosing diabetic retinopathy and a value of 190.8 with sensitivity of 56.1%, Specificity of 64.6% for diagnosing diabetic nephropathy (Table 4.11).

Table 4. 11: Diagnostic accuracy of biochemical markers for microvascular complications

Threshold values	AUC	Sensitivity (95% CI)	Specificity (95% CI)	PPV	NPV	TP	TN	FP	FN	Accuracy	p-value
Retinopathy											
FBS											
6.5mmol/l	0.5219	92.6% (75.3 to 98.9)	21.9% (14.3 to 32.1)	28.10%	90.00%	25	18	64	2	0.3945	0.7246
HBA1c (%)											
9.30%	0.5339	25.9% (13.0 to 45.0)	91.4% (83.0 to 96)	50.00%	78.95%	7	75	7	20	0.7523	0.6266
Sialic acid(mg/dl)											
205.4mg/dl	0.5824	48.1% (30.8 to 65.9)	79.3% (69.1 to 86.6)	43.33%	82.28%	13	65	17	14	0.7156	0.2490
Nephropathy											
FBS (mmol/l)											
9.5mmol/l	0.4845	41.5% (27.8 to 56.7)	69.5% (58.8 to 78.0)	40.00%	70.00%	17	57	25	24	0.601	0.7869
HBA1c (%)											
5.30%	0.3415	100.0% (89.5 to 100)	2.4% (0.2 to 9.0)	34.00%	100.00%	41	2	80	0	0.349	0.0012
Sialic acid											
190.8mg/dl	0.5730	56.1% (41.0 to 70.1)	64.6%(53.8 to 74)	44.00%	75.00%	23	53	29	18	0.618	0.1783

4.16 MULTIVARIATE LOGISTIC REGRESSION ANALYSIS FOR MICROVASCULAR COMPLICATIONS USING METABOLIC VARIABLES AND SIALIC ACID

The odds of developing retinopathy in diabetes was very high for WHR (OR= 4.39, P=0.060), HDL (OR= 5.73, P=0.152), LDL (OR= 2.53, P=0.214), TG (OR= 1.19, P=0.858), HbA1c (OR= 1.035, P=0.842) and significantly low for DBP (OR= 0.90, P=0.021) (Table 4.12)

In addition, the odds of developing diabetic nephropathy were significantly low for WHR (OR= 1.89 x 10^{-6}, P=0.0001), HbA1c (OR= 0.617, P=0.042), DBP (OR= 0.920, P=0.039) and high for SBP (OR= 1.055, P=0.008) (Table 4.13).

Table 4.12: Age-adjusted multivariate Logistic regression analysis for retinopathy using metabolic variables and sialic acid

Variables	Adjusted Odds Ratio	Lower 95%	Upper 95%	p-values
HipC	1.003	0.910	1.107	0.9475
W/H ratio	4.386	1.184	5.836	0.0598
VAI	1.439	0.425	4.791	0.5502
BAI	0.912	0.768	1.074	0.2776
SBP	1.001	0.951	1.050	0.9477
FBS	1.013	0.888	1.150	0.8415
HBA1c	1.035	0.732	1.460	0.8427
T CHOL	0.403	0.059	1.388	0.2368
TRIG	1.193	0.155	8.121	0.8584
HDL	5.728	0.582	78.65	0.1519
LDL	2.532	0.774	16.68	0.2143
SSA	1.005	0.999	1.012	0.0820
DBP	0.901	0.820	0.979	**0.0207**

Table 4.13: Age-adjusted multivariate Logistic regression analysis for nephropathy using metabolic variables and sialic acid

Parameters	Adjusted Odds Ratio	Lower 95%	Upper 95%	p-value
HipC	1.066	0.979	1.168	0.1487
WHR	1.89x 10-^{6}	1.35x10-^{9}	0.001	**0.0001**
VAI	1.450	0.865	4.253	0.4791

BAI	1.035	0.890	1.211	0.6486
SBP	1.055	1.018	1.104	**0.0076**
FBS	0.983	0.859	1.118	0.7965
HBA1c	0.617	0.374	0.955	**0.0416**
T CHOL	0.275	0.043	1.133	0.1343
TRIG	1.024	0.145	5.420	0.9796
HDL	1.598	0.159	18.20	0.6981
LDL	2.854	0.710	17.905	0.2181
SSA	1.001	0.994	1.008	0.6275
DBP	0.920	0.844	0.990	**0.0390**

4.17 GLYCEMIC INDICES, MEASURE OF ADIPOSITY, HYPERTENSION, LEVEL OF INFLAMMATORY PARAMETERS AND DYSLIPIDEMIA AMONG DIABETIC PATIENTS WITH AND WITHOUT MICROVASCULAR COMPLICATIONS.

Among the diabetic patients, 45.3% (68) presented with microvascular complications and associated increase in blood glucose and decrease in glycated haemoglobin levels (Table 4.14). Control of blood glucose was poor in 47.1% and good in 52.9% of the patients with diabetic microvascular complications. Hypertension grade 1 (SBP=140159/DBP=90-99) was observed in 16.2% and grade 3 in 1.5% of the diabetics with microvascular complications (Table 4.14). The diabetic patients with microvascular complication had increased in levels of sialic acid and decrease in CRP, however there was no significant differences (P> 0.05). Waist circumference, WHR adiposity indices and BMI showed no significant differences (P>0.05) between the patients with and without microvascular complications. However, 45.6%, 29.4% of those with microvascular complications were overweight and obese respectively (Table 4.14).

Dyslipidemia was observed in greater proportion with hypercholesterolemia in 45.6%, hypertriglyceridemia in 7.4% and increased LDL in 38.2% of the patients with microvascular complications.

Table 4.14: Glycemic indices, Measure of Adiposity, Hypertension, level of inflammatory parameters and dyslipidemia among diabetic patients with and without microvascular complications Variables Microvascular complication

	No	Yes	P-value
	(n= 82)	(n= 68)	
FBG (mmol/l)	9.25 ± 3.57	9.48 ± 4.14	0.714
HBA1c (%)	7.59 ± 1.34	7.29 ± 1.42	0.182
Glycaemic control			0.021
Poor	54 (65.9)	32 (47.1)	
Good	28 (34.1)	36 (52.9)	
Blood Pressure Categories			0.546
Optimal	5 (6.1)	8 (11.8)	
Normal	22 (26.8)	14 (20.6)	
Prehypertension	0 (0.0)	0 (0.0)	
Hypertension	9(11.0)	12 (17.7)	
Grade 1	9 (11.0)	11 (16.2)	
Grade 2	0 (0.0)	0 (0.0)	
Grade 3	0 (0.0)	1 (1.5)	
Inflammatory parameters SSA			
(mg/dL)	185.55 ± 75.69	210.09 ± 98.25	0.086
CRP	0.16 ± 0.05	0.06 ± 0.02	0.098
WC (cm)	92.56 ± 15.46	92.29 ± 10.14	0.900
WHR	0.90 ± 0.07	0.90 ± 0.07	0.934
Adiposity indices			
VAI	1.52 ± 0.81	1.97 ± 0.43	0.253
BAI	32.64 ± 9.77	32.55 ± 4.87	0.947
BMI n (Kg/m2)	28.90 ± 6.33	28.12 ± 4.97	0.405
BMI n (%)			0.120
Underweight	2 (2.4)	1 (1.5)	
Normal	24 (29.3)	16 (23.5)	
Overweight	22 (26.8)	31 (45.6)	
Obese	34 (41.5)	20 (29.4)	
Lipid profile			
TC (>5.2 mmol/L)	39 (47.6)	31 (45.6)	0.809

TG (>1.8 mmol/L)	7 (8.5)	5 (7.4)	0.790
HDL-CHL (<1.1mmol/L)	19 (23.2)	16 (23.5)	0.959
LDL-CHL (>4.2 mmol/L)	22 (26.8)	26 (38.2)	0.136

DISCUSSION

5.1 AGE AND GENDER RELATION WITH T2DM

Advanced age shows triple risks of developing T2DM as compared to younger ages. Globally, the largest proportion of people with T2DM is between 40-59 years (Whiting *et al.*, 2011) which was also reflected in this study where the mean age of the diabetics was 58.90 ±12.43. The high proportion of females (61.3) in this study may be due to the nature of population admitting to this hospital in that more of them seek medical attention than men (38.7%) and the fact that diabetes is more prevalent in females than males (Crook *et al.*, 1994; Amoah *et al.*, 2000). The largest percentage (29.3%) of diabetic patients in this study was found in the age group, 60 – 69 years. However, a sizeable percentage (21.3%), were ≥70 years. Only a low percentage (7.3%) were below 40 years, a value that is lower compared to a value of

13.0% obtained for type 2 diabetic patients <40 years (Aguilar-Salinas *et al.*, 2003). In sub-Saharan Africa, prevalence of diabetes increases with age, with most reports indicating a peak at either 65 years or older (Ahren & Corrigan, 1984; Ducorps *et al.*, 1996; Fichtlscherer *et al.*, 2000) or 55–64 years (Mollentze *et al.*, 1995). Age seems to be a relevant risk factor for diabetes and association suggests that, in Africa, the effect of ageing of the population on diabetes prevalence is already evident (Elbagir *et al.*, 1996; Christensen *et al.*, 2009).

The observation of more females (61.3%) than males (38.7%) with diabetes in the present study is consistent with the observation by Wild *et al.* (2004), that there were more women with diabetes mellitus than men. The present study also recorded more

elderly women than men (Table 4.3). The combined effect of a greater number of elderly women than men in most populations, and the increasing prevalence of diabetes mellitus with age is the most likely explanation for this observation. This pattern, however, confirms that the prevalence of diabetes mellitus increases with age for both males and females; further in developing countries, the majority of people with diabetes mellitus are in the 45 – 64 years range (King *et al.*, 1998; Hillier & Pedula, 2001; Wild *et al.*, 2004). In Nigeria, Ekpenyong *et al.* (2012) also found diabetes to be higher among females than males.

5.2 ANTHROPOMETRIC VARIABLES IN T2DM

In most studies from sub-Saharan Africa, adiposity (encompassing body-mass index, waist and hip circumference, and waist-to-hip ratio and adiposity indices) has generally been associated with diabetes. Data indicate that prevalence of the disorder rises with increasing body-mass index, waist-to-hip ratio, and waist circumference (Cooper *et al.*, 1997; Welborn *et al.*, 2003; Motala *et al.*, 2008). Significant differences in the mean waist circumference VAI and BAI between diabetic patients and non-diabetics were observed. Mean BMI and waist-to-hip ratio was not significantly different between diabetic patients and non-diabetics, however overweight and obesity was prevalent in the diabetic patients for the present study. These results corroborate the findings in several studies where high overweight and obesity prevalence were recorded in patients with diabetes (Kaushik, 2006; Nguyen *et al.*, 2008; Oghagbon *et al.*, 2009). It was revealed that 36.0% of the diabetic patients were obese, with a BMI ≥30 kg/m^2 whilst 35.3% were overweight with a BMI 25.0 –

29.9 kg/m^2. Thus, 71.3 % were overweight or obese or had a BMI ≥25.0 kg/m^2, which is lower than a value of 75.0% overweight or obese type 2 diabetic patients reported in Mexico (Aguilar-Salinas *et al.*, 2003). Researchers from South Africa (Levitt *et al.*,

1993; Motala *et al.*, 2008), reported very high rates of obesity (58–65%) in individuals with diabetes compared with people from Tanzania (9·1%) and Sudan (7·7%).

The mean BMI of males (26.98 ± 5.20 kg/m2) and females (29.54 ± 5.88 kg/m2) both indicate overweight, however, the females have a significantly higher BMI (P = 0.007) than males. Similarly, the percentage of female diabetics who were obese (74.1%) and overweight (62.3%) were significantly higher than the corresponding values of 25.9% and 37.7% for male diabetics (P = 0.021). This is consistent with earlier results by Akbar (2002) that indicated that obesity was more common in females than males in type 2 diabetic patients. Females have been known to be more prone to abdominal obesity compared with their male counterparts due to their vulnerability. Women who were nutritionally deprived in childhood are more likely to be obese in adulthood, while men who were deprived in childhood face no greater risk. On the average, women have more body fat than men. This could be attributed to impact of eostrogen as it reduces their ability to burn energy after eating which results in increase storage of fat in the body (Stephen, 2007; Ekpenyong *et al.*, 2012). Obesity characterized by excess body fat is probably the most notable risk factor for the development of type 2 diabetes (Edelstein *et al.*, 1997; Wild *et al.*, 2004). This, however, could account for the higher prevalence of obesity, increase in waist circumference, waist-to-hip ratio and adiposity indices (VAI and BAI) in the diabetic females in this study. Thus, a higher percentage of Ghanaian female diabetics (61.3%) than males (38.7%) as a result of obesity and type 2 diabetes were evident. These results corroborate the findings in several studies where high overweight and obesity prevalence were recorded in female patients with diabetes (Kaushik, 2006; Oghagbon *et al.*, 2009; Mitolo *et al.*, 2015).

5.3 METABOLIC VARIABLES (BLOOD PRESSURE AND DYSLIPIDEMIA) IN T2DM

Type 2 diabetes is associated with devastating complications usually classified as microvascular (retinopathy, nephropathy, and neuropathy) and macrovascular (coronary vascular disease, stroke, and peripheral vascular disease). Cardiovascular disease (CVD) complications are far more common than microvascular complications and are the leading cause of death and disability in type 2 diabetes (Shantaram, 1999). Multiple risk factors are associated with CVD in type 2 diabetic patients, including hypertension, hyperlipidaemia and obesity (Haffner *et al.*, 2000). These risk factors are also the main features of the metabolic syndrome. Persons with elevated blood pressure are 2.5 times more likely to develop diabetes mellitus within 5 years (Sowers & Bakris, 2000) than persons without either condition. This is because the coexistence of hypertension and diabetes mellitus is particularly pernicious because of strong linkage of the two conditions with cardiovascular disease (Fagan & Sowers, 1999; Haffner *et al.*, 2002). This is evident in the present study with significant increase in mean systolic and diastolic pressures among the diabetics (SBP≥ 120mmHg, P= 0.001; DBP≥80, P=0.022) compared with the non-diabetics. Gender difference in blood pressure was not significant; however, SBP and DBP were increased in the females than the men. Several coexisting factors have been attributed to females developing hypertension since they are more prone to obesity, which is a common denominator to developing diabetes and hypertension (Sowers *et al.*, 2000).

Dyslipidemia as a metabolic abnormality is frequently associated with diabetes mellitus. Abnormalities in lipid metabolism have been reported in patients with diabetes mellitus accompanied by the risk of cardiovascular arteriosclerosis

(Goldberg, 2001; Krauss, 2004). The lipoprotein abnormalities commonly present in T2DM include hypertriglyceridemia and reduced plasma HDL cholesterol. In the present study, higher mean serum levels of total cholesterol, triglycerides and LDL cholesterol with low HDL were noted in patients with diabetes, which are well known risk factors for cardiovascular diseases among patients, when compared to the patients with no diabetes (Table 2). This therefore supports the fact that, defects in insulin action and hyperglycemia could lead to changes in plasma lipoproteins in patients with diabetes (Ginsberg, 1996; Taghibiglou *et al.*, 2000).

Among the patients with diabetes, levels of serum total cholesterol, triglycerides and LDL cholesterol and HDL-cholesterol were increased in the females than the males. This could also be associated with the increase in adiposity in the females since obesity has been widely associated with dyslipidemia (Krauss, 2004; Langat, 2011).

5.4 COMPLICATIONS ASSOCIATED WITH T2DM

The common causes of diabetic complications are poor control of diabetes either due to non-adherence, poor attitude towards the disease and its complications, unhealthy diet, and insufficient physical activity, and due to poor management by the health care professionals (Fitzgerald *et al.*, 1995; Ajayi & Ajayi, 2009; Sharma *et al.*, 2011). Among the diabetic patients, 45.3% (68) presented with microvascular complications. A review by Bos and Agyemang (2013) indicated varying prevalence of chronic diabetes complications in northern Africa (Herman *et al.*, 1998; Macky *et al.*, 2011). The high prevalence of nephropathy (27.3%) followed by retinopathy (18%), which are the most specific complication of hyperglycaemia, suggests a delay between the onset of diabetes and the time of diagnosis (Harzallah *et al.*, 2006; Christensen *et al.*, 2009). In one study in Egypt, about 80% of the patients lacked the knowledge about the ocular hazards of diabetes (Macky *et al.*, 2011). In the patients with diabetes, nephropathy

(29.3%) was prevalent in those between the ages of 70-79 years and retinopathy (44.3%) in the patients aged 60-69 years. The high incidence of diabetic complications at age's ≥ 60 years may suggest a direct relationship, in that a diabetic patient is more likely to develop nephropathy and retinopathy at old age. Recent studies have reported that poor renal function is a risk factor for falls in older adults (Dukas *et al.*, 2005; Kengne *et al.*, 2005). Gender relation showed 68.3% and 31.7% nephropathy in the females and males respectively and 66.7% and 33.3% retinopathy in the females and males respectively. Blood pressure was noticed to be higher in the diabetics with nephropathy and lower in those with retinopathy than the diabetics with no complications. High rates of microvascular complications are at least partly attributable to frequent high blood pressure and inappropriate diabetes control, in relation to limited access to care.

Overall, retinopathy affects 15–55% of patients, with a high proportion of proliferative retinopathy and macular oedema. In individuals with type 2 diabetes, 21– 25% have retinopathy at diagnosis of diabetes compared with 9·5% of those with type 1 diabetes (Mbanya *et al.*, 2010). In cohorts with mean diabetes duration of 5–10 years, 32–57% has microalbuminuria or macroalbuminuria, and a third to half of people on maintenance haemodialysis have diabetes (Mbanya & Sobngwi, 2003). Coronary heart disease can affect 5–8% of individuals with type 2 diabetes and cardiomyopathy—up to 50% of all patients with type 2 diabetes (Kengne *et al.*, 2005).

5.4.1 Metabolic variables (Blood pressure and Dyslipidemia) in T2DM patients with and without complications

In patients with T2DM, many studies have clearly established that complications are mainly due to chronic hyperglycemia that exerts its health effects through several mechanisms: hypertension, dyslipidemia, platelet activation, and altered endothelial

metabolism (Jokl & Colwell, 1997; Brownlee, 2001). In light of this study, grade 1 hypertension (SBP=140-159/DBP=90-99) was observed in 16.2% and grade 3 (SBP=≥ 180/DBP=≥ 110) in 1.5% of the diabetic patients with microvascular complications. CVD accounts for up to 80% of the deaths in persons with type 2 diabetes (Haffner *et al.*, 1990). Population-based studies showed that CVD mortality was 7.5 times greater among persons with type 2 diabetes without a previous myocardial infarction than in those without diabetes (Haffner *et al.*, 1990; Sowers *et al.*, 2000).

Dyslipidemia was observed in greater proportion with hypercholesterolemia in 45.6%, hypertriglyceridemia in 7.4% and increased LDL in 38.2% of the patients with microvascular complications in this study. Previous studies have however established increased levels of cholesterol and triglycerides in diabetic patients that have developed complications with comparable occurrence in the patients with diabetic retinopathy and nephropathy (Estacio *et al.*, 2000; Avogaro *et al.*, 2007). Lehto *et al.* (1997) observed that high calculated LDL cholesterol level (≥4.1 mmol/l) was significantly associated with all CHD events. The simultaneous presence of high fasting glucose (>13.4 mmol/l) with low HDL cholesterol, low HDL–to–total cholesterol ratio, or high total triglycerides further increased the risk for CHD events up to threefold (Lehto *et al.*, 1997). The association between lipidemia and CHD could partly account for the complications observed in the type 2 diabetic patients since CHD/CVD have been established as determinant of diabetic complications (Mykkanen *et al.*, 1993; Bloomgarden, 2003).

5.5 GLYCATED HEMOGLOBIN AND GLYCEMIC CONTROL IN T2DM

This study showed significantly (P=0.001) increased glycated haemoglobin levels in type-2 diabetics compared with non-diabetics. These findings were in accordance to the study of (Khurshid *et al.*, 2010). HbA1c is formed by a non-enzymatic irreversible

process with combination of aldehyde group of glucose and the amino terminal valine of β chain of haemoglobin. As plasma glucose is consistently elevated, there is increase in non-enzymatic glycation of haemoglobin (Chen *et al.*, 1996; Ahmad, 2005) hence the increase in HbA1c observed in this study.

Poor self-care ability was significantly related to poor glycemic control in the study by carolin et al 1997. Previous studies have used measures of metabolic control either to assess validity of self-care scales (Davis & Lewis, 1991) or to focus on the relationship of diabetes attitudes (Andersen *et al.*, 1983; Wilson *et al.*, 1986), or provide relationship to self-care practices and self-care ability. It was not possible to study in cross-sectional data whether patient attitudes toward their diabetes and patient self-care ability were associated with discouragement with the degree of control of hyperglycemia or an admission that self-care was a problem (Street *et al.*, 1993; Harris, 1996). This finding could partly account for the higher percentage of poor glycemic control (57.3%) among the diabetics. Moreover, measures of adiposity and central obesity were also increased in the patients with poor control resulting
40.7% becoming obese.

The findings were in line with a previous study suggesting that the level of total cholesterol is usually normal or near normal if glycaemic control is adequate, and worsening of control raises the level (Andersen *et al.*, 1983).

Further in this study is control of glycaemia in the patients that have developed diabetic complications. Control of blood glucose was poor in 47.1% and good in 52.9% of the patients with diabetic microvascular complications and a decrease in glycated haemoglobin compared to those without microvascular complications was observed.

5.6 INFLAMMATORY MARKERS IN T2DM

The metabolic syndrome affects at least one quarter of the population in developed countries and is associated with an increased risk of developing type 2 diabetes and cardiovascular diseases (Jacobs *et al.*, 2009). This study showed a significant graded increase of serum sialic acid and C-reactive protein in the diabetics with the lowest levels in non-diabetic subjects. Serum sialic acid is considered as a marker of innate immunity and activated innate immunity is a risk factor for cardiovascular mortality in type 2 diabetes (Nigam *et al.*, 2006). Low grade inflammation has been hypothesized to underlie the pathogenesis of the metabolic syndrome and its association with cardiovascular diseases (Pischon *et al.*, 2008). One important determinant of low-grade inflammation in the metabolic syndrome might be central obesity, because visceral adipose tissue may produce inflammatory mediators, which in turn induce the production of acute phase reactants in hepatocytes and endothelial cells (Baars *et al.*, 2013). This could also account for the increase in higher SSA and CRP in the female diabetics in this study. Indeed, the metabolic syndrome has been associated with markers of inflammatory activity, such as C-reactive protein (CRP), interleukin 6 (IL6), serum amyloid A (SAA) and soluble adhesion molecules (Jacobs *et al.*, 2009; Kressel *et al.*, 2009). This observation of increased plasma concentrations of CRP indicates a greater global systemic inflammation status in diabetic patients, which has been previously suggested that may reflect the activity of the underlying atherosclerotic process (Baars *et al.*, 2013), and it is concordant with several crosssectional studies which have shown an increase of CRP levels in patients with diabetes (Festa *et al.*, 2002; Wannamethee *et al.*, 2007).

The association of Type 2 diabetes mellitus and metabolic syndrome with increased SSA could possibly be due to generalized endothelial cell dysfunction or macrovascular disease, either through loss of sialic acid containing glycoproteins from vascular cells into the blood stream or through an acute phase response (Rahman *et al.*, 2010). The diabetic process stimulates cytokine production from cells throughout the body, and these cytokines play a direct role in the causation of vascular complication. Furthermore, insulin is known to be an anabolic hormone and one of its actions is to increase certain acute-phase proteins, it may be that insulin resistance in diabetes and metabolic syndrome causes an acute-phase protein response alternatively there may be abnormalities of sialylation of glycoproteins and glycolipids in the metabolic syndrome (Crook *et al.*, 1994). The increased serum sialic acid in this study is in concordance with the results of previous studies (Pickup, 2004; Rahman *et al.*, 2011).

5.6.1 Inflammatory markers in diabetic patients with and without microvascular complications

Research studies have shown that the concentration of sialic acid in serum is elevated in pathological states when there is tissue damage, tissue proliferation and inflammation. Hence extensive microvascular damage associated with diabetes mellitus, could account for its shedding into the circulation leading to an increase in vascular permeability and overall increased SSA concentrations (Crook *et al.*, 2001; Gopaul & Crook, 2006). In the present study, serum sialic acid levels were increased in diabetic patients with complications with higher values in those with retinopathy followed by nephropathy compared with levels in diabetic patients with no complications. Tissue injury caused by diabetic vascular complications stimulates local cytokine secretion from cellular infiltrates such as macrophages and endothelial cells. This induces an acute phase response with release of acute phase glycoproteins with

sialic acid from the liver into the general circulation again leading to increased SSA concentrations (Crook *et al.*, 2001).

The findings in this study are similar to that by Nayak and Bhaktha (2005) where decreased sialic acid levels in type-2 diabetics without any complications and increase in type-2 diabetic with nephropathy, when compared. C-reactive protein, on the other hand was higher in diabetics with no complication and lower in those with complication with the least in the diabetic patients with retinopathy.

5.7 RELATIONSHIP BETWEEN MARKERS OF INFLAMMATION (SSA AND CRP), FBG AND GLYCEMIC CONTROL, ANTHROPOMETRIC VARIABLES AND DYSLIPIDEMIA

The cause and effect relationship between inflammatory markers and glycemic control is still unclear i.e., whether poor glycemic control leads to inflammation or whether inflammation leads to higher glucose levels (or whether a third factor influences both). King *et al.* (2004) suggested per the finding of their study that, if poor glycemic control leads to inflammation, then better glycemic control should lower inflammation and therefore lower the risk of cardiovascular complications. The low CRP levels observed in the diabetic patients with good control are consistent with such finding, contrary to that is high levels of SSA in diabetics with good control in the present study. Furthermore, if inflammation leads to poor glycemic control, then treatment of inflammation with NSAIDs or hydroxymethylglutaryl-CoA reductase inhibitors may help improve glycemic control. Findings in this study are consistent with those of previous studies in that higher adiposity indices, waist circumference and WHR were associated poor glycemic control in the diabetic patients with obesity in 40.7% (Benoit *et al.*, 2005; Fox *et al.*, 2006).

It has been shown that in the individuals with impaired glucose tolerance (Müller *et al.*, 2002), the low-grade chronic inflammation is related to glucose metabolic disturbance and a growing body of evidence supports the hypothesis that chronic systemic inflammation contributes to decrease insulin sensitivity. Moreover, hyperglycemia is a significant stressor that has also been shown to cause chronic inflammation (Crook *et al.*, 2001; Pickup, 2004). However, the relationship between inflammatory markers and glycemic control is still not fully understood. In this study, the inflammation markers (SSA and CRP) were not significantly correlated with FBG and HbA1c levels. This result is consistent with the findings of Lindberg *et al.* (1991) indicating that hyperglycemia is unlikely to have a major effect on the acute phase response in type 2 diabetes. Serum sialic acid levels showed significant correlation with the degree of metabolic control (as estimated by HbA1c) as reported by (Yasutada *et al.*, 1978; Festa *et al.*, 2000; Gopaul *et al.*, 2006). However, previous cross-sectional studies which have found an inconsistent association between inflammation and blood glucose levels (Yasutada *et al.*, 1978; Crook *et al.*, 2001), possibly due to previous treatment with metformin, statins and antihypertensive agents, which may limit the power to detect an association between HbA1c and inflammatory markers. In light of previous findings of an association among inflammatory proteins, endothelial dysfunction, and insulin resistance (Yudkin *et al.*, 1999; Fichtlscherer *et al.*, 2000), the results of the current study provide additional support for a relation between glycemic control and systemic inflammation in people with established diabetes.

Positive significant correlation was found between CRP, BMI and BAI; SSA with WHR though not significant. These are similar to the finding of other studies (Haffner *et al.*, 2000; Browning *et al.*, 2004) . Central adiposity is a cardiovascular risk factor and this may explain the correlation observed with Serum Albumin with BMI WHR and

Adiposity indices (Festa *et al.*, 2002). This further supports the theory that SSA can be used as a marker for cardiovascular risk (Yudkin *et al.*, 1999).

In addition, non-significant inverse relationships were observed for both inflammatory markers with TC, TG and LDL in the present study. Contrary these findings, serum sialic acid levels showed significant correlation with cardiovascular risk factors such as cholesterol, LDL and TG (Festa *et al.*, 2000; Haffner *et al.*, 2000). Apolipoproteins contain Sialic acid, which may influence the behaviour of the associated lipoproteins, and changes in sialylation of LDL particles in particular have been linked with atherosclerosis. Increased SA content of VLDL particles may contribute to hypertriglyceridaemia. Good correlation was also observed between sialic acid and important cardiovascular risk factors such as cholesterol, LDL and TG by (Festa *et al.*, 2000; Nayak *et al.*, 2005).

5.8 RISK FACTORS ASSOCIATED WITH POOR CONTROL IN T2DM

In light of the present study, gender, age and obesity were determined to increase the odds of poor glycemic control. Study in an emerging group of diabetic patients, specifically patients younger than 40 years, identified them as a group at high risk for diabetes complications, because of their poor glycemic control. Change in weight and use of pharmacologic therapy, which are considered major determinants and targets in relation to glycemic control were also observed (El-Kebbi *et al.*, 2003). This is consistent with diabetes clinic findings (Dabelea *et al.*, 1999; Duncan, 2006). Diabetic complications showed low risk with nephropathy being a low significant factor associated with poor glycemic control.

5.9 RISK FACTORS ASSOCIATED WITH DEVELOPING MICROVASCULAR COMPLICATIONS IN TYPE 2 DIABETES

The baseline cross-sectional data in the study by Tesfaye *et al.* (2005) are evidence of a strong association between neuropathy and other microvascular complications, suggesting a common pathogenic mechanism (Cameron *et al.*, 2001; Eaton *et al.*, 2003). Several of the risk factors associated with microvascular complications in this study are markers of insulin resistance. Both an elevated triglyceride level and obesity are strong predictors of microalbuminuria and retinopathy in type 2 diabetes (Chaturvedi *et al.*, 2001). A potential link between insulin resistance and microvascular complications including neuropathy is their association with endothelial dysfunction (Zenere *et al.*, 1995). Other complications of diabetes linked to endothelial dysfunction may also predict the risk of microalbuminuria, retinopathy, and neuropathy (Zenere *et al.*, 1995; Cameron *et al.*, 2001).

5.10 PREDICTIVE VALUE OF FBS, HBA1C AND SIALIC ACID IN THE DIAGNOSIS OF DIABETIC MICROVASCULAR COMPLICATIONS.

Fasting plasma glucose and HbA1c were observed to be fair tests for the diagnosing diabetic retinopathy and poor for diagnosing diabetic nephropathy. However sialic acid was observed in this study to be a good diagnostic marker for microvascular complications (retinopathy and nephropathy) although with low sensitivity. Measurement of inflammation sensitive markers may be useful for assessment of the cardiovascular risk in diabetic patients. Results from prospective studies suggest that inflammation is involved in the pathogenesis of diabetes (Ford, 2005) and atherosclerosis (Ross, 1999). Inflammation could be a common antecedent for both diabetes and cardiovascular disease. Hyperglycaemia and insulin resistance could also promote inflammation, and may be factor linking diabetes to the development of

atherosclerosis. Elevated glucose levels could promote inflammation by increased oxidative stress (Baynes & Thorpe, 1999). Sialic acid maintains the negative charge of renal glomerular basement membrane, which is one of the main regulators of membrane permeability. Due to increased vascular permeability there is shedding of vascular endothelial sialic acid leading to its increased levels in circulation (Prajnab *et al.*, 2013).

Chapter 6

CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

Results from this study indicated direct association of HbA1c with elevation of SSA and CRP. These may imply a significant relation between inflammation and glycemic control in people with established diabetes. Monitoring serum sialic acid levels in diabetic patients may help in predicting the subclinical inflammatory process in them. In addition, the present study confirms that low-grade chronic inflammation in T2DM is related to LDL and obesity.

In addition, findings of this study are that elevated serum sialic acid was strongly related to the presence of microvascular complications like nephropathy and retinopathy in type 2 diabetic patients. Therefore, estimation of sialic acid levels may help in early prediction and prevention of microvascular complications occurring due to diabetes mellitus type-2, thereby decreasing the mortality and morbidity.

6.2 RECOMMENDATION

Prospective studies should be conducted to determine the direction of this association; such research would have important implications for the treatment of adults with diabetes.

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