KWAME NKRUMAH UNIVERSITY OF SCIENCE & TECHNOLOGY,

KUMASI

COMPARISON OF GLYCATED ALBUMIN AND GLYCATED HEMOGLOBIN LEVELS IN THE MANAGEMENT OF TYPE 2 DIABETIC PATIENTS IN THE TEMA METROPOLIS OF GHANA

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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

Background: Glycated Albumin (GA) has been proposed as an important index in assessing chronic glycaemic control in diabetic patients other than glycated hemoglobin (HbA1c), besides it reflects shorter periods of glycaemia.

Aim: This study sought to validate the use of Glycated Albumin and Glycated Hemoglobin, as biomarkers of glycaemic control among Ghanaian diabetes patients. **Research design and methods**: Venous blood samples were taken from 200 participants of whom 150 were type 2 diabetic patients and 50 healthy individuals without diabetes. The blood samples were analyzed for fasting glucose, lipid profile, renal function (BUN, CRT and eGFR), serum total protein and albumin on fully automated analyzer, Roche COBAS Integra 400 Plus System. The A_1 fast fraction – cation exchange method was used to estimate the level of glycated hemoglobin whilst the sandwich enzyme-linked immunosorbent one-step process assay (ELISA) was used for the assay of the Human Glycosylated Albumin (GA) level in the samples. **Results**: Blood glucose, Glycated hemoglobin, Glycated albumin, GA/HbA1c and serum albumin were significantly ($P< 0.05$) increased in the patients with diabetes compared to the non-diabetics. Renal assessment indicated significant differences ($P< 0.05$) in levels of serum urea, creatinine and sodium with increased levels in the diabetic patients. The estimated glomerular filtration rate (eGFR) was also significantly ($P<0.0001$) reduced in the diabetics (eGFR value) compared to the non-diabetes (eGFR value). The proportion of excellent control of blood glucose assessed using GA, 11.3%, was lower than that assessed by HbA1c (16.7%). Also, glycemic control assessed by GA showed a greater proportion of poor control (35.3%) than when assessed by HbA1c (28.7%). Across the various age groups, diabetic nephropathy (29.3%) was more prevalent in the diabetic patients aged between 70-79 years and retinopathy (43.3%) more prevalent in the patients aged 60-69 years. Correlation between the levels of HbA1c and Glycated albumin among patients with diabetes a showed a highly significant relationship ($P<0.001$). Poor glycaemic control determined by HbA1c and GA were highly associated with Obesity and reduced kidney function.

Conclusion: Glycated albumin reflects glucose excursions more strongly than HbA1c; hence GA might be a more sensitive index for some diabetic complications than HbA1c.

DEDICATION

I dedicate this work to God Almighty whose grace and mercy has brought me this far and also to my lovely husband George Bortie, my son Caleb, my daughters Rachael and Stephanie, my mother Emelia Ameley Okine and father Emmanuel Ayeequaye Okine.

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ABBREVIATIONS

AGE:	Advanced glycation end products
ADP:	Adenosine-5-diphosphate
ATP:	Adenosine-5-triphosphate
BUN:	Blood urea nitrogen
BMI:	Body mass index
BCG:	Bromocresol green
°C :	Degrees Celsius
CKD:	Chronic Kidney Disease
CKD- EPI:	Chronic Kidney Disease Epidemiology Collaboration
cm:	Centimeter
CAT-I:	Carnitine acyl transferase
DM:	Diabetes Mellitus
DKA:	Diabetic ketoacidosis
DHAP:	Dihydroxyacetone phosphate
EDTA:	Ethylene diamine tetracetic acid
ELISA:	Enzyme-Linked Immunosorbent Assay
eGFR:	Estimated Glomerular Filtration Rate
FBG :	Fasting blood glucose
FFAs:	Free fatty acids

GA :	Glycated Albumin
GDM:	Gestational diabetes mellitus
g :	grams
GLDH:	Glutamate dehydrogenase
GFR:	Glomerular Filtration Rate
HbA:	Hemoglobin A
HbS :	Hemoglobin S
HbC:	Hemoglobin C
HbD:	Hemoglobin D
HB:	Hemoglobin
HK:	Hexokinase
HbA1C/GHb:	Glycated Haemoglobin
HHS:	Hyperosmolar hyperglycaemic state
HDL:	High density lipoprotein
2Hr PP:	Two (2) hour post prandial
H_2O_2:	Hydrogen peroxide
IFG :	Impaired Fasting Glycaemia
IGT:	Impaired Glucose Tolerance
IEC:	International Expert Committee
IR:	Insulin Receptor
Kg:	kilogram
Kg/m^2:	kilogram per meters squared
KDOQI:	Kidney Disease Outcomes Quality Initiative
KNUST:	Kwame Nkrumah University of Science & Technology
LADA:	Latent Autoimmune Diabetes of Adults
LDL:	Low Density Lipoprotein

LDL-C:	Low density lipoprotein cholesterol concentration
Mmol/L:	Millimol per liter
ml:	Mills
NCEP ATP III:	National Cholesterol Education Programme Adult Treatment Panel III
NH3:	Ammonia
OGTT:	Oral Glucose Tolerance Test
RBG:	Random blood glucose
SCD:	Sickle cell disease
S.ALB:	Serum Albumin
TC:	Total cholesterol
TP:	Total Protein
TG:	Total triglyceride
TGH	Tema General Hospital
T2DM:	Type2 Diabetes Mellitus
VLDL:	Very low density lipoprotein
WHO:	World Health Organization
WHR:	Waist to Hip Ratio
WC:	Waist Circumference

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Diabetes mellitus is a cluster of metabolic disorders whose main characteristic is persistent hyperglycaemia. Type 1 diabetes mellitus (T1DM) is largely due to cellular mediated autoimmune destruction of the β-cells of islets of langerhans and results in decreased insulin production. Type 2 diabetes (T2DM) is characterized by insulin resistance or abnormal insulin secretion (Kumar *et al.*, 2005).

An estimated 2.8% of the world's population has diabetes and this is expected to increase to about 4.4% by the year 2030. T2DM however makes up about 90% of the cases (Hedley *et al.*, 2004; Wild *et al.*, 2004). In Ghana, type 2 diabetes mellitus is at a crude prevalence rate of 6.3% and an age-adjusted prevalence of 6.4% (Amoah *et al.*, 2002).

With the rapidly increasing prevalence and projections on diabetes mellitus, there is an urgent need to develop affordable and effective preventive strategies and identify highrisk populations in whom such strategies can be implemented (King *et al.*, 1998).

Various laboratory tests are available for screening and monitoring or managing diabetic cases. These include: fasting or random blood glucose (FBG/RBG), oral glucose tolerance test (OGTT), two (2) hour post prandial test and glycated haemoglobin (HbA1c). HbA1c, the most widely used assay, measures the percentage of circulating haemoglobin that has chemically reacted with glucose and reflects blood glucose concentrations over the prior 120 days, with the most profound effect in the preceding 30 days (Calisti & Tognetti, 2005). It therefore reflects the ability of

metabolic control within the desired range and also enables the estimation of the risk of chronic diabetic complications (Takahashi *et al.*, 2007).

This has however come with its own problems and limitations. HbA1c values are influenced significantly in all conditions or haemoglobinopathies characterized by either shortening of the life span of erythrocytes or the changing proportion of young to old erythrocytes (Goldstein *et al.*, 2004). Some of these include, haemoglobin variants (HbS, HbC, HbD), drugs, anaemia, uremia, alcoholism and dialysis. Limitations resulting from most inherent assay methods also compromise the clinical utility of the HbA1c maker (Calisti *et al.*, 2005).

A newer indicator of glycaemic control, the Glycated Albumin (GA) has been proposed and is rapidly becoming a significant index in assessing glycaemic control. Early stage reaction product of albumin or serum protein is called Glycated Albumin or Fructosamine (Ahmad, 2005). It has been suggested that GA provides a significantly better glycaemic control in patients with conditions that may cause decreased red cell lifespan, especially haemodialysis. Assessment of HbA1C in such patients is likely to cause an underestimation mostly due to increasing proportions of young erythrocytes (Inaba *et al.*, 2007).

Moreover, serum albumin has a shorter lifespan (15-20days) than that of red blood cells (120 days) and with a higher turnover than haemoglobin. This also makes GA better in assessing short-term changes (2 weeks) in diabetic control. There have also been suggestions on the need for a mid-range test that could be performed monthly as a means of helping people with diabetes manage their glucose levels more effectively (Takahashi *et al.*, 2007).

In the light of these benefits, GA may also be affected by other factors like BMI, treatment modalities and endogenous insulin secretion. There is therefore the need to also evaluate other factors which may affect the interpretation and use of this marker.

Moreover the characteristics and applicability of this marker in the Ghanaian population remains to be determined. The current study therefore seeks to evaluate the use of Glycated Albumin compared to HbA1C as biomarkers of glycaemic control and other factors which may be associated with its use.

1.2 Study Hypothesis

Plasma GA is a more accurate marker of glycaemic control than HbA1c which could help in the determination of short term glucose control, hence better management of diabetic patients.

1.3 Problem Statement

Glycated hemoglobin has over the years been a very useful tool in the monitoring of glycaemic control in diabetics. This has however come with several challenges. The test is relatively expensive and is affected by several conditions, which may decrease red blood cell survival, a pre-requisite for adequate chemical bonding of glucose. This therefore is an indication of the non-applicability of the test in all populations, hence the need to identify a better marker of glycaemic control for every population. Glycated Albumin has therefore been suggested. There are reports that, Glycated Albumin is also affected by endogenous insulin secretion in diabetics(Koenig *et al.*, 1976), thus necessitating the evaluation of the marker, especially among type 2 diabetes patients.

1.4 Justification

Internationally, at least one person dies every 10 seconds whereas four limbs are surgically remove every 30 seconds as a result of diabetic complications. Prevalence

and projections on Diabetes Mellitus is alarming and there is an urgent need to develop affordable and effective preventive strategies (King *et al.*, 1998). The numerous complications that present with diabetes will also be prevented or reduced drastically upon effective strategies to diagnose, predict and manage the condition.

The HbA1c test is designed to measure the average blood glucose levels over previous 2-3 months, giving an indicator of longer-term blood glucose control(Calisti *et al.*, 2005). Aside the high cost of performing the test, which is largely borne by patients, the test comes with various limitations, which make it unreliable and inappropriate for monitoring glycaemic control(Goldstein *et al.*, 2004). Some of these include, haemoglobin variants (HbS, HbC,HbD), drugs, anemia, uremia, alcoholism and dialysis.Limitations resulting from most inherent assay methods also compromise the clinical utility of the HbA1c maker(Calisti *et al.*, 2005). The characteristics of GA and HbA1c in a cross-section of diabetic patients have not been compared in Ghana. Hence we have no justification yet, to include or adopt this as a marker of glycaemic control in Ghana.

There is therefore the need to conduct such a study in Ghana, which will add to existing database on diabetes by providing baseline information. The study will assess the ability of Glycated Albumin to better demonstrate glycaemic control and management. It will also determine which factors may be associated with these levels in the Ghanaian population.

1.5 Expected Benefits

Success in proving our hypothesis will help reduce cost incurred by patients on HbA1c analysis, better detect changes in glycaemic control and thus early prevention of the onset of complications. It will also identify factors, which may affect the levels of

Glycated Albumin among the Ghanaian population, hence, the better interpretation in the use of the marker in management.

1.6 Aim

The aim of the study was to validate the use of Glycated Hemoglobin and Glycated Albumin, as biomarkers of glycaemic control among Ghanaian diabetic patients.

1.6.1 Specific objectives

- To compare Glycated Hemoglobin and Glycated Albumin as biomarkers of glycaemic control.
- To determine the effects of anthropometric variables on Glycated Albumin levels
- To determine the relationship between Glycated Albumin and dyslipidemia in diabetics.
- To determine the relationship between glycaemic control and complications associated with Diabetes Mellitus

CHAPTER 2

LITERETURE REVIEW

2.0 Diabetes

Diabetes mellitus is a disorder of insulin deficiency or resistance that is characterized by hyperglycaemia and is associated with imbalance in carbohydrate, protein, and fat metabolism(Davis & Lewis, 1991). Diabetes is a chronic non-communicable debilitating disease that requires life-long treatment and greatly increases the risk of serious, longterm complications namely blindness, kidney disease, and neural, vascular damage leading to foot ulcers (World Health Organization, 2006) which may require amputation and also increase the tendency to heart attack, stroke and early death

(Motala, 2010). Neuropathy (nerve damage) can also be caused by diabetes in which the individual or patient experiences numbness or weakness in the hands or feet and the development of foot ulcers, which may eventually lead to limb amputation.

Overall, death risk among people with diabetes is twice as that of people of the same age who do not have diabetes (Langat, 2011).

According to the World Health Organization (WHO) the current diagnostic criteria for diabetes are: 1) plasma glucose concentration measured after an overnight fast above 7.0mmol/l and/or 2) plasma glucose concentration measured two hours after a 75g oral glucose load above 11.0mmol/l (Gavin III *et al.*, 1997).

There are two main types that have been diagnosed in patients globally namely Type 1 diabetes and Type 2 diabetes. Diabetes happens when the pancreas does not produce adequate insulin, or when the body is not able to effectively use the insulin it produces.

Diabetes mellitus (Type 1 diabetes) also known as insulin-dependent diabetes mellitus or juvenile-onset diabetes results from autoimmune mediated destruction of the beta cells of the pancreas (Norris *et al.*, 2001). Blood glucose must be regulated with insulin treatment in combination with a balanced diet and physical exercise. If the level of glucose falls too low, hypoglycaemia can lead to unconsciousness. If the blood glucose level remains too high (hyperglycaemia), the body breaks down fat reserves instead of glucose as an energy source, giving rise to the release of toxic ketones and acids (ketoacidosis), which can lead to coma and death. At present, there is no way of preventing type 1 diabetes, and people diagnosed with it must receive insulin treatment for life. Insulin resistance often also precedes type 2 diabetes: the body produces insulin but the tissue cells do not respond fully; more and more insulin is produced until insulin production fails and blood glucose rises (Preeth *et al.*, 2014).

Type 2 diabetes also known as non–insulin-dependent diabetes mellitus or adult-onset diabetes is characterized by resistance to the action of insulin and disorder of insulin secretion, either of which may be the predominant feature (Preeth *et al.*, 2014). Type 2 diabetes can be controlled through a balanced diet and exercise plus (usually at a later stage) oral anti-diabetic drugs. Insulin is also increasingly used to treat type 2 diabetes, and evidence is increasing that early insulin treatment has a significant effect in delaying or preventing complications (Turner *et al.*, 1998). One particular form – ketosis-prone atypical diabetes – is mainly found in people of African origin. It involves severe hyperglycaemia and ketoacidosis but can be controlled with insulin treatment (Mbanya *et al.*, 2010). Type 2 diabetes, which is the most common type, is often a result of excess body weight and physical inactivity in genetically predisposed individuals (Shojania *et al.*, 2006).

Gestational diabetes mellitus (GDM) which is another type found in only women comes as result of pregnancy. It is a form of glucose intolerance diagnosed during the second or third trimester of pregnancy. Mothers with GDM and their babies are at risk of developing types 2 diabetes mellitus if proper care is not ensured (Calisti & Tognetti, 2005; Clausen *et al.*, 2008). GDM is known to be increasing in prevalence. Recent reports indicates that it affects 3-15% of pregnancies worldwide(Kapur, 2011)

Type 2 diabetes accounts for over 90% of diabetes cases in Sub-Saharan Africa (Levitt, 2008), whilst Type 1 diabetes, gestational diabetes, and variant forms such as atypical ‘ketosis-prone’ diabetes and malnutrition-related diabetes constitute the remainder.

Prediabetes is a condition in which a person's blood glucose levels are higher than normal but not high enough for a diagnosis of type 2 diabetes. Many people destined to develop type 2 DM spend many years in a state of prediabetes which has been termed "America's largest healthcare epidemic (Wild *et al.*, 2004).

Latent autoimmune diabetes of adults (LADA) is a condition in which type 1 DM develops in adults. Adults with LADA are frequently initially misdiagnosed as having type 2 DM, based on age rather than etiology(Balkau *et al.*, 2002).

Some cases of diabetes are caused by the body's tissue receptors not responding to insulin (even when insulin levels are normal, which is what separates it from type 2 diabetes); this form is very uncommon. Genetic mutations (autosomal or mitochondrial) can lead to defects in beta cell function. Abnormal insulin action may also have been genetically determined in some cases. Any disease that causes extensive damage to the pancreas may lead to diabetes (for example, chronic pancreatitis and cystic fibrosis). Diseases associated with excessive secretion of insulin-antagonistic hormones can cause diabetes (which is typically resolved once the hormone excess is removed). Many drugs impair insulin secretion and some toxins damage pancreatic beta cells (Alberti & Zimmet, 1998).

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%
Prevalence	~10%	~90%

2.1 Signs and Symptoms

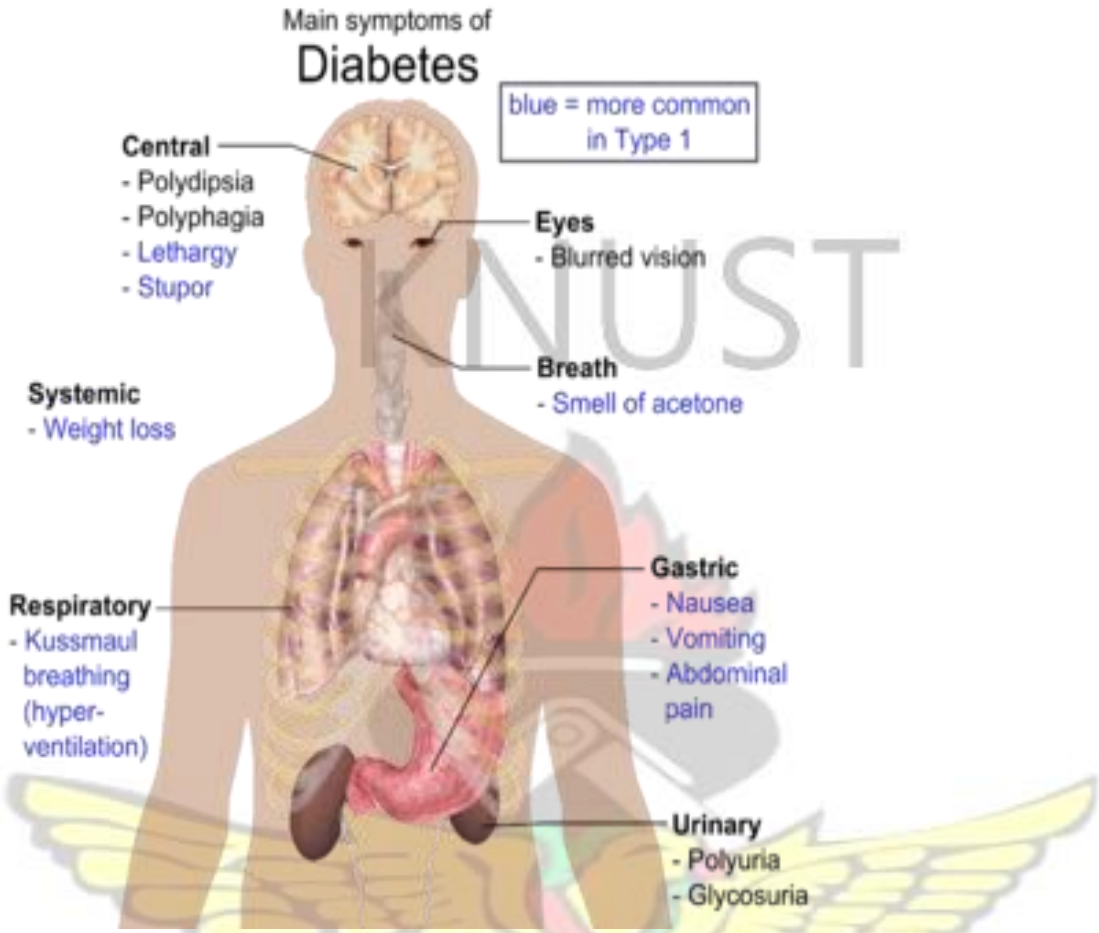


Figure 2.1: Signs and symptoms of Diabetes*(Williams textbook of endocrinology, 2000)*

The classic symptoms of untreated diabetes are loss of weight, polyuria (frequent urination), polydipsia (increased thirst), and polyphagia (increased hunger). Symptoms may develop rapidly (weeks or months) in type 1 diabetes, while they usually develop much more slowly and may be subtle or absent in type 2 diabetes (Cooke, 2008).

Prolonged high blood glucose can cause glucose absorption in the lens of the eye, which leads to changes in its shape, resulting in vision changes. Blurred vision is a common complaint leading to a diabetes diagnosis. A number of skin rashes that can occur in diabetes are collectively known as diabetic dermatomes (Pickup, 2004).

2.2 Causes of Diabetes

The cause of diabetes depends on the type.

2.2.1 Type 1

Type 1 diabetes was previously called insulin dependent diabetes mellitus or juvenileonset diabetes. Although the disease onset can occur at any age, the peak age for diagnosis is in the mid-teens (Cooke, 2008). This condition is partly inherited, and in genetically susceptible people, the onset of diabetes can be triggered by one or more environmental factors, such as a viral infection (Coxsackie B4 virus) or diet. Type 1 diabetes develops when the cells that produce the hormone insulin, known as the beta cells, in the pancreas are destroyed. This destruction is initiated or mediated by the body's immune system and limits or completely eliminates the production and secretion of insulin, the hormone that is required to lower blood glucose levels (McLarty *et al.*, 1990; Goldstein *et al.*, 2004).

To survive, people with type 1 diabetes must have insulin delivered by injection or a pump. In adults, type 1 diabetes accounts for approximately 5% of all diagnosed cases of diabetes (Motala, 2010). There is no known way to prevent type 1 diabetes. Several clinical trials for preventing type 1 diabetes are currently in progress with additional studies being planned. Unlike type 2 diabetes, the onset of type 1 diabetes is unrelated to lifestyle. Type 1 diabetes can be accompanied by irregular and unpredictable hyperglycemia, frequently with ketosis, and sometimes with serious hypoglycemia (Levitt, 2008). Other complications include an impaired counter regulatory response to hypoglycemia, infection, gastro paresis (which leads to erratic absorption of dietary carbohydrates), and endocrinopathies (e.g., Addison's disease). These phenomena are believed to occur no more frequently than in 1% to 2% of persons with type 1 diabetes (Levitt, 2008; Clausen *et al.*, 2008).

2.2.2 Type 2

Type 2 diabetes was previously called non–insulin dependent diabetes mellitus or adultonset diabetes because the peak age of onset is usually later than type 1 diabetes. In adults, type 2 diabetes accounts for about 90% to 95% of all diagnosed cases of diabetes. Type 2 diabetes usually begins with insulin resistance, a disorder in which the cells primarily within the muscles, liver, and fat tissue do not use insulin properly (Cooke, 2008).

As the need for insulin rises, the beta cells in the pancreas gradually lose the ability to produce sufficient quantities of the hormone. The role of insulin resistance as opposed to beta cell dysfunction differs among individuals, with some having primarily insulin resistance and only a minor defect in insulin secretion, and others with slight insulin resistance and primarily a lack of insulin secretion (Stratton *et al.*, 2000; Goldstein *et al.*, 2004).

The risk for developing type 2 diabetes is associated with older age, obesity, family history of diabetes, and history of gestational diabetes, impaired glucose metabolism, physical inactivity, and race/ethnicity. Excess body fat is associated with 30% of cases in those of Chinese and Japanese descent, 60-80% of cases in those of European and African descent, and 100% of Pima Indians and Pacific Islanders. Those who are not obese often have a high waist–hip ratio (Kuzuya & Matsuda, 1997).

Dietary factors also influence the risk of developing type 2 diabetes. Consumption of sugar-sweetened drinks in excess is associated with an increased risk (Malik *et al.*, 2010). The type of fats in the diet is also important, with saturated fats and trans fatty acids increasing the risk and polyunsaturated and monounsaturated fat decreasing the risk (Risérus *et al.*, 2009). Eating lots of white rice appears to also play a role in

increasing risk and lack of exercise is believed to cause 7% of cases (Hu & Stampfer, 2003; Lee *et al.*, 2003).

2.3 Diagnosis of Diabetes Mellitus

The clinical diagnosis of diabetes mellitus is often prompted by symptoms such as polyuria, polydipsia, recurrent infections, unexplained weight loss, and in severe cases, drowsiness and coma (Umpierrez *et al.*, 1997). High levels of glycosuria are usually present. A single random (casual) blood glucose estimation in excess of the diagnostic values (venous plasma ≥11.1 mmol/L, venous whole blood ≥10.0 mmol/L) establishes the diagnosis in such cases. The report also defines levels of random blood glucose below which a diagnosis of diabetes mellitus is unlikely in non-pregnant individuals (venous plasma <5.5 mmol/L, venous whole blood <4.4 mmol/L) (American Diabetes Association, 2005).

For clinical purposes, an oral glucose tolerance test (OGTT) to establish diagnostic status needs only be considered if casual blood glucose values lie in the uncertain range, that is, between the levels that establish or exclude diabetes mellitus (venous plasma ≥5.5 and <11.1 mmol/L, venous whole blood ≥4.4 and <10.0 mmol/L) and fasting blood glucose levels are below those which establish the diagnosis of diabetes mellitus but above the upper reference limit. If an OGTT is performed, it is sufficient to measure the blood glucose values whilst fasting and at 2 hours after a 75g oral glucose load. For children the oral glucose load is related to body weight: 1.75g per kg (Alberti *et al.*, 1998; W.H.O, 2006; ADA, 2006).

The values of Impaired Fasting Glycaemia (IFG) are a fasting venous plasma glucose concentration of 6.1 mmol/L or greater (venous whole blood 5.6 mmol/L), but less than 7.0 mmol/L (venous whole blood 6.1mmol/L); and if a 2-hour post glucose is measured,

a fasting venous plasma glucose concentration of less than 7.8 mmol/L (venous whole blood 6.7 mmol/L) (Alberti, 1996).

The values for Impaired Glucose Tolerance (IGT) are a fasting venous plasma 2-hour post glucose concentration of 7.8 mmol/L or greater (venous whole blood 6.7 mmol/L), but less than 11.1mmol/L (venous whole blood 10.0 mmol/L); and if a fasting glucose is measured, a fasting venous plasma glucose concentration of less than 7.0 mmol/L (venous whole blood 6.1 mmol/L)(Alberti, 1996).However, for clinical purposes, the diagnosis of diabetes mellitus should always be confirmed by repeating the test on another day unless there is unequivocal hyperglycaemia with acute metabolic decompensation or obvious symptoms as recommended by the expect committee (Alberti, 1996; Mberti, 1998) .

Glucose concentrations should not be determined on serum, unless red cells are immediately removed, otherwise glycolysis will result in an unpredictable under estimation of the true concentrations. Glucose preservatives do not totally prevent glycolysis. Thus, if whole blood is used, the sample should be kept at 0 – 4 ^{0}C, or assayed immediately. If plasma is used, the blood sample should be centrifuged immediately (Alberti *et al.*, 1998).

An alternative to blood glucose estimation or the OGTT has long been sought to simplify the diagnosis of diabetes mellitus. Glycated haemoglobin (HbA1C) reflecting average glycaemia over the preceding 2─3 months was thought to provide such a test. Although in certain cases it gives equal or almost equal sensitivity and specificity to glucose measurement (McCance *et al.*, 1994), it is not available in many parts of the world and is not well enough standardized for its use to be recommended now. However HbA1C is currently considered the best index of metabolic control for diabetic patients in clinical settings (Goldstein *et al.*, 2004; Nathan *et al.*, 2005) and participants in

epidemiological studies as well as a measure of risk for the development of micro- and macrovascular complications. For population studies of glucose intolerance and diabetes, individuals have been classified by their blood glucose concentration measured after an overnight fast and/or 2 hours after a 75g oral glucose load. Since, it may be difficult to be sure of the fasting state, and because of the strong correlation between fasting and 2-hour values, epidemiological studies or diagnostic screening have in the past been restricted to the 2-hour values only. Whilst this remains the single best choice, if it is not possible to perform the OGTT (e.g. for logistical or economic reasons), the fasting plasma glucose alone may be used for epidemiological purposes (Alberti *et al.*, 1998; Crowther *et al.*, 2005; Krolewski *et al.*, 2014).

To determine if gestational diabetes mellitus is present in pregnant women, a standard OGTT should be performed after overnight fasting (12 – 14 hours) by giving 75g anhydrous glucose in 250-300ml water. Plasma glucose is measured at fasting and 2 hours after glucose intake. Pregnant women who meet the WHO criteria for diabetes mellitus or IGT are classified as having gestational diabetes mellitus (GDM). After the pregnancy ends, the woman should be reclassified as either having diabetes mellitus, or IGT, or normal glucose tolerance based on the results of a 75g OGTT six weeks or more after delivery (Alberti *et al.*, 1998; Kim *et al.*, 2002).

2.4 Glycated Hemoglobin

Epidemiological studies have confirmed that hyperglycaemia is the most important factor in onset and progress of diabetes complications, both in T1DM and type T2DM. Mechanisms connecting hyperglycaemia with long term complications of diabetes have been investigated. Among others, a large number of useful proofs indicated the involvement of non-enzymatic glycation processes (Lyons & Jenkins, 1997). Nonenzymatic glycation is the process by which glucose is chemically bound to amino

groups of amino acids of proteins. 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 non-enzymatic 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 end products (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. α-oxoaldehydes 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 auto-oxidation 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 non-enzymatic 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 non-enzymatically 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 end-products. 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. 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 NH2-terminal 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 glycaemic control (Bucala & Cerami, 1992; Brownlee, 2000).

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 & Shima, 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 (Nathan *et al.*, 1984; Goldstein, 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 programs including the American Diabetes Association to assess the quality of diabetes care (Davidson, 1998). HbA1C

concentration is also related to prevalent coronary disease or carotid intimal thickening in non-diabetic 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 HbA1C goal of <7% (Lawson *et al.*, 1999; Stratton *et al.*, 2000).

2.5 Glycated Albumin

Glycated albumin (GA) is known to reflect short-term glycaemic levels, and could be a useful therapeutics monitor in DM because the half-life of albumin (17 days) is shorter than that of erythrocytes (28 days) (Yamada *et al.*, 2008) . Several studies (Guthrow *et al.*, 1979; Koga *et al.*, 2010) have shown that GA is a more reliable DM monitor and a better marker of glycaemic control than is HbA1c in patients undergoing hemodialysis and in patients with fluctuating and poorly controlled type 2 DM. Moreover, serum GA is not affected by factors that affect haemoglobin metabolism (Suwa *et al.*, 2010). The International Expert Committee (IEC) recently proposed a new diagnostic criteria based on measurement of HbA1c (Kilpatrick *et al.*, 2009). However, little attention has been paid to the utility of GA estimation compared with that of HbA1c in the diagnosis of DM. The GA assay is not widely available and is not standardized; thus, there is only very limited data to suggest that it would be useful as a diagnostic tool. In this study, our aim was to establish the validity of GA as a measure of glycaemic control and to evaluate its utility as a diagnostic tool for DM in a community-based.

Measurements of glycated haemoglobin (HbA1C) and glycated albumin (GA) have been used clinically to monitor glycaemic control in patients with diabetes. A1C represents an integrated measurement of blood glucose during the preceding 2 months while serum GA, a shorter-term marker, reflects glycaemic control over approximately the preceding 2 weeks (Guthrow *et al.*, 1979; Shima *et al.*, 1988).

GA is not influenced by a number of physiologic and pathologic conditions that affect HbA1C levels, such as anemia and genetic haemoglobin abnormalities (Bry *et al.*, 2001).Unfortunately, there may also be interferences with the GA assay. While HbA1c measurement is affected by reduced erythrocyte survival or an increase in young erythrocytes (e.g., during treatment with erythropoietin stimulating agents), GA can be influenced by factors that affect albumin turnover (Koga *et al.*, 2007; Miyashita *et al.*, 2007). Because the majority of patients with advanced nephropathy have overt proteinuria, GA values may also be affected in these patients. One study has shown this to be the case; there was a significant decrease in GA values independent of glycaemic state in diabetic patients with nephritic syndrome, while non-nephrotic range proteinuria did not significantly influence GA(Okada *et al.*, 2011).

Since the half-life of serum albumin is around 2 weeks, shorter than that of erythrocytes, GA reflects shorter terms of glycaemic control than HbA1c (Tahara *et al.*, 1993). Reflecting such characteristics, it has been recently shown that changes in GA can predict change in HbA1c after diabetes treatment (Okada *et al.*, 2011; Won *et al.*, 2012).In addition, there have been accumulating evidences that HbA1c mainly reflects mean plasma glucose levels while GA also reflects plasma glucose excursions and/or postprandial glucose levels better than HbA1c (Cohen, 1988; Ogawa *et al.*, 2012).

2.6 Complications of Diabetes Mellitus

All forms of diabetes increase the risk of long-term complications. These typically develop after many years (10–20), but may be the first symptom in those who have otherwise not received a diagnosis before that time.

The major long-term complications relate to damage to blood vessels. Diabetes doubles the risk of cardiovascular disease and about 75% of deaths in diabetics are due to coronary artery disease (Sarwar *et al.*, 2010; O'Gara *et al.*, 2013). Other "macrovascular" diseases are stroke, and peripheral vascular disease.

The primary microvascular complications of diabetes include damage to the eyes, kidneys, and nerves. Damage to the eyes, known as diabetic retinopathy, is caused by damage to the blood vessels in the retina of the eye, and can result in gradual vision loss and potentially blindness Damage to the kidneys, known as diabetic nephropathy, can lead to tissue scarring, urine protein loss, and eventually chronic kidney disease, sometimes requiring dialysis or kidney transplant. Damage to the nerves of the body, known as diabetic neuropathy, is the most common complication of diabetes (Christensen *et al.*, 2009). The symptoms can include numbness, tingling, pain, and altered pain sensation, which can lead to damage to the skin. Diabetes-related foot problems (such as diabetic foot ulcers) may occur, and can be difficult to treat, occasionally requiring amputation. Additionally, proximal diabetic neuropathy causes painful muscle wasting and weakness.

There is a link between cognitive deficit and diabetes. Compared to those without diabetes, those with the disease have a 1.2 to 1.5-fold greater rate of decline in cognitive function (Cukierman *et al.*, 2005).

2.7 Metabolic effects of Insulin and Diabetes Mellitus

Insulin is the principal hormone responsible for the control of glucose metabolism. It is synthesized by the β-cells of the islets of Langerhans of the pancreas as a precursor, pro-insulin, which is processed to form C-peptide and insulin. Both are secreted in equimolar amounts into the portal circulation (Domanski & Proschan, 2004). The mature insulin molecule comprises two polypeptide chains, the A chain and the B chain (21 and 30 amino acids respectively). The two chains are linked together by two interchain disulphide bridges (A7 to B7 and A20 to B19). There is also an intra-chain disulphide bridge in the A chain (connects residues 6 and 11). Secretion of insulin is mainly controlled by plasma glucose concentration and the hormone has a number of important metabolic actions. Its first principal function is to control the uptake and utilization of glucose in peripheral tissues via the glucose transporter. This and other hypoglycaemic activities, such as the inhibition of hepatic gluconeogenesis and glycogenolysis are counteracted by the hyperglycaemic hormones including glucagon, epinephrine (adrenaline) growth hormone, thyroxine and cortisol(Kahn, 2003). Insulin concentrations are severely reduced in type 1 diabetes mellitus and some other conditions such as hypopituitarism. Insulin levels are relatively raised in type 2 diabetes mellitus, obesity, insulinoma and some endocrine dysfunctions such as Cushing's syndrome and acromegaly. Insulin signaling at the target tissue results in a large array of biological outcomes. These events are essential for normal growth and development and for normal homeostasis of carbohydrate, lipid and protein metabolism. Elucidating the intracellular events after activation of the insulin receptor (IR) has been the primary focus of a large number of investigators for decades, and for excellent reasons. Numerous prospective studies in various populations indicate that insulin resistance and insulin secretory dysfunction predict the development of type 2 diabetes (Ferrannini,

1998; Weyer *et al.*, 2001)and are therefore targets for primary prevention of the disease. Understanding the signaling pathways involved in insulin action could lead to a better understanding of the pathophysiology of insulin resistance associated with type 2 diabetes mellitus and obesity. Identifying associated key molecules and processes could lead to newer and more effective therapeutic agents for treating these common disorders.

2.8 Metabolic Complications of Diabetes Mellitus

Diabetic ketoacidosis (DKA) and hyperosmolar hyperglycaemic state (HHS) are the two most serious acute metabolic complications of diabetes mellitus, even if managed properly. These disorders can occur in both type 1 and type 2 diabetes respectively. The mortality rate in patients with diabetic ketoacidosis is <5%, whereas the mortality rate of patients with hyperosmolar hyperglycaemic state (HHS) is about 15% (Hamblin *et al.*, 1989; Basu *et al.*, 1992). The prognosis of both conditions is substantially worsened at the extremes of age and in the presence of coma and hypotension (Malone *et al.*, 1992). DKA consists of the biochemical triad of hyperglycaemia, ketonaemia and acidaemia. The degree of hyperglycaemia in DKA is quite variable and may not be a determinant of the severity of DKA. In HHS there is more severe hyperglycaemia and hyperosmolality than DKA. HHS may consist of variable degrees of clinical ketosis as determined by the nitroprusside method and may often present without coma. Serum osmolality has been shown to correlate significantly with mental status in DKA and HHS (Ennis *et al.*, 1994; Umpierrez *et al.*, 1997).

Although the pathogenesis of DKA is better understood than that of HHS, the basic underlying mechanism for both disorders is a reduction in the net effective concentration of circulating insulin (Polonsky *et al.*, 1994), coupled with concomitant elevation of counter regulatory stress hormones, such as glucagon, epinephrine

(adrenaline), growth hormone, thyroxine and cortisol. Thus DKA and HHS are extreme manifestations of impaired carbohydrate regulation that can occur in diabetes mellitus (Umpierrez *et al.*, 1997) Although many patients manifest overlapping metabolic clinical pictures, each condition can also occur in relatively pure form. In patients with DKA, the deficiency in insulin can be absolute, or it can be insufficient relative to an excess of counter regulatory hormones. In HHS, there is a residual amount of insulin secretion that minimizes ketosis but does not control hyperglycaemia. This leads to severe dehydration and impaired renal function leading to decreased excretion of glucose (Ennis *et al.*, 1994). These factors coupled with the presence of a stressful condition result in more severe hyperglycaemia than that seen in DKA. In addition, inadequate fluid intake contributes to severe hyperosmolality, the hallmark of HHS.

The most common precipitating factor in the development of DKA or HHS is infection (Basu *et al.*, 1992). The most common types of infections are pneumonia and urinary tract infections, accounting for 30−50% of cases.

Other acute medical illnesses which are precipitating causes include alcohol abuse, trauma, pulmonary embolism, myocardial infarction, and pancreatitis, which can occur both in type 1 and type 2 diabetes (Nathan *et al.*, 2005).Various drugs that alter carbohydrate metabolism, such as corticosteroids, pentamidine, sympathomimetic agents, and α- and β-adrenergic blockers, and excessive use of diuretics in the elderly may also precipitate the development of DKA and HHS.

Psychological factors and poor compliance, leading to omission of insulin therapy, are important precipitating factors for recurrent ketoacidosis. In young female patients with type 1 diabetes, psychological problems complicated by eating disorders may be contributing factors in up to 20% of cases of recurrent ketoacidosis (Polonsky *et al.*, 1994; Rydall *et al.*, 1997). Factors that may lead to insulin omission in younger patients

include fear of weight gain with good metabolic control, fear of hypoglycemia, rebellion against authority and stress related to chronic disease (Polonsky *et al.*, 1994). Non-compliance with insulin therapy has been found to be the leading precipitating cause for DKA in urban African-Americans and medically indigent patients (Musey *et al.*, 1995; Umpierrez *et al.*, 1997).

2.9 Lipid and Ketone Metabolism

The increased production of ketones in DKA is the result of a combination of insulin deficiency and increased concentrations of counter regulatory hormones, particularly epinephrine, which lead to the phosphorylation and activation of hormone-sensitive lipase in adipose tissue(McGarry, 1979; Jensen *et al.*, 1989; Nurjhan *et al.*, 1992). The increased activity of tissue lipase causes a breakdown of triglyceride into glycerol and free fatty acids (FFAs). Although glycerol is used as a substrate for gluconeogenesis in the liver and the kidney, the massive release of FFAs assumes pathophysiological predominance in the liver, where the FFAs serve as precursors of the ketoacids in DKA (McGarry, 1979; DeFronzo *et al.*, 1994). In the liver, FFAs are oxidized to ketone bodies, a process predominantly stimulated by glucagon. Increased concentration of glucagon in DKA reduces the hepatic levels of malonyl-CoA by blocking the metabolism of pyruvate to acetylCoA through inhibition of acetyl-CoA carboxylase, the first rate-limiting enzyme in de novo fatty acid synthesis(Gerich *et al.*, 1976; McGarry, 1979; Nurjhan *et al.*, 1992) Malonyl-CoA inhibits carnitine acyl transferase I (CAT-I), the rate limiting enzyme for transesterification of fatty acyl-CoA to fatty acyl-carnitine, regulating oxidation of fatty acids to ketone bodies. CAT-I is required for movement of FFA into the mitochondria, where fatty acid oxidation takes place. The increased fatty acyl-CoA and CAT-I activity in DKA, lead to increased ketogenesis in DKA (McGarry *et al.*, 1989; Zammit, 1994). In addition to increased production of

ketone bodies, there is evidence that clearance of ketones is decreased in patients with DKA (Reichard *et al.*, 1986; Balasse & Fery, 1989). This decrease may be due to low insulin concentration, increased glucocorticoid level and decreased glucose utilization by peripheral tissues(Nosadini *et al.*, 1989). Epinephrine secretion by the adrenal medulla is markedly enhanced in DKA. In vitro, epinephrine has a marked effect to increase lipolysis in adipocytes. In vivo, epinephrine can increase plasma concentrations of FFAs, at least when insulin deficiency is present. In addition, epinephrine facilitates hepatic ketogenesis directly (Avagaro *et al.*, 1993). Norepinephine at concentrations that approximate those seen in the synaptic cleft stimulates lipolysis by adipocytes and enhances ketogenesis(Keller *et al.*, 1984).In addition to the individual effects of stress hormones, infusion of combinations of counter regulatory hormones has been observed to have synergistic effects when compared with those seen with single hormone infusions (Shamoon *et al.*, 1981).

The risks associated with the metabolic syndrome, as is currently conceived is 30-50% for diabetes, 12-17% for cardiovascular disease and about 6-7% for all-cause mortality (Ford, 2005). Beyond CVD and type 2 diabetes, individuals with metabolic syndrome seemingly are susceptible to other conditions, notably polycystic ovary syndrome, fatty liver, cholesterol gallstones, asthma, sleep disturbances and some forms of cancer. NCEP ATPIII (Grundy, 2002) identified 6 components of the metabolic syndrome that relate to CVD: Central (abdominal) obesity, atherogenic dyslipidemia, raised blood pressure, insulin resistance and glucose intolerance, pro-inflammatory state and prothrombotic state. Abdominal obesity is the form of obesity most strongly associated with the metabolic syndrome. It presents clinically as increased waist circumference. Atherogenic dyslipidemia manifests in routine lipoprotein analysis as raised triglycerides and low concentrations of HDL cholesterol (Rubins, 2000). A more

detailed analysis usually reveals other lipoprotein abnormalities, e.g. increased remnant lipoproteins, elevated apolipoprotein B, small LDL particles, and small HDL particles. All of these abnormalities have been implicated as being independently atherogenic. Elevated blood pressure strongly associates with obesity and commonly occurs in insulin resistant persons. Patients with longstanding insulin resistance frequently manifest glucose intolerance, another emerging risk factor. When glucose intolerance evolves into diabetes-level hyperglycemia, elevated glucose constitutes a major, independent risk factor for CVD (Grundy *et al.*, 2004; Ford, 2005).

CHAPTER 3

MATERIALS AND METHODS

3.1 Study Site and Study Design

This case comparative study was conducted at the Diabetic Clinic of the Tema General Hospital, the Eye Clinic and the Chemical Pathology Department of the Tema General Hospital Laboratory. The Tema General Hospital is the main referral center within the Tema Metropolis; as such it provided adequate participants required for the study**.**

3.2 Study Population

A total of 200 participants were recruited for this study of which 150 were known and confirmed diabetics and 50 were healthy individuals attending the Hospital. The 150 diabetics consisted of: 79 diabetics without any complications, 41 with diabetic renal damage and 30 with diabetic retinopathy.

3.2.1 Sample Size Justification

The following formula was used:

$$t^2 \times P\ (1 - P)$$

$$N = \frac{t^2 P}{m}$$

Where;

N= sample size, t=confidence interval of 95% (standard value of 1.96), **P**= prevalence rate (6.4%), **m**=margin of error (standard value of 0.05). Hence **N= 92 (minimum).** A sample size of **200** was thus chosen for this study

3.2.2 Inclusion Criteria

Participants must be Ghanaians above the age of 18 years, and should have been diagnosed with type 2 diabetes mellitus and on medication (i.e. on insulin and/or diet with oral hypoglycaemic drugs), diagnosed using the WHO criteria. The controls included Ghanaian participants with normal glucose tolerance, assessed using WHO criteria, which involved an oral glucose tolerance test, with an absence of diabetes mellitus within first-degree relatives. Participants must be in good health, to qualify as controls, b) with Type 2 diabetes and with no complications, c) Type 2 diabetes with nephropathy d) Type 2 diabetes with retinopathy.

3.2.3 Exclusion Criteria

Non-Ghanaian diabetic patients, persons with type 1 diabetes, persons with recent or chronic conditions that could affect concentrations of inflammatory markers (eg. cancer), persons taking cholesterol-lowering medication, diabetic pregnant women who were not physiologically normal, acutely ill diabetic patients, too ill to be interviewed, or those with severe medical conditions were excluded from the study. Non Ghanaian control participants, non-diabetic control participants with diabetes within first degree relatives, abnormal glucose tolerance, and heavy smokers (more than one pack of cigarettes per day) were also excluded from the study.

3.3 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 Ethical Consideration

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

3.5 Data Collection

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 participants included presence of diabetes within first-degree relatives.

3.6 Anthropometric Measurement

For both diabetics and controls, body weight and height were measured using a standard physician's scale and standiometer, to the nearest 0.1 kg and 0.5 cm respectively, with participants in lightweight clothing without shoes and standing in an upright position. BMI was calculated as weight/height^2 (kg/m^2). Waist circumference was measured with a plastic anthropometric tape on bare skin of standing subjects during mid-respiration

at the narrowest indentation midway between the lowest rib and the iliac crest and at the level of the umbilicus. It was measured to the nearest 0.1 cm. Duplicate measures were made and averages were used in the analysis.

3.7 Blood Sample Collection and Processing

About 5 ml of venous blood sample was collected from the antecubital fossa of the study participants after an overnight fast (10 – 12 hours). One milliliter (1 ml) of the blood sample was dispensed into fluoride oxalate tube. About 1 ml of the blood sample was dispensed into ethylene diamine tetraacetic acid (EDTA) tube and the other 3 ml into vacutainer plain tubes. Serum was stored at -20C after centrifugation at 500rpm for 15 minutes until assay was performed. Assay parameters included: fasting blood glucose (FBG), glycated hemoglobin and albumin, total protein, serum albumin, blood urea nitrogen (BUN), creatinine, sodium, potassium, total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL) cholesterol. Serum low density lipoprotein (LDL) cholesterol was estimated from the Friedewald equation (Friedewald *et al.*, 1972). The assay was performed on the biochemistry autoanalyzer, Roche COBAS Integra® 400 Plus System (Roche Diagnostics, Germany, West Berlin) with the system's respective reagent cassettes.

3.8 Assay Methods

3.8.1 Fasting Blood Glucose

Glucose concentration in the samples was estimated with the hexokinase method. Hexokinase (HK) phosphorylates glucose with ATP to produce glucose-6-phosphate, which was then oxidized by glucose-6-phosphate dehydrogenase to 6phosphogluconate with the simultaneous reduction of NAD^+ to NADH. The resulting increase in absorbance at 340nm was directly related to the concentration of glucose in the sample.

$$Glucose + ATP \overset{HK}{\rightarrow} Glucose - 6 - phosphate + ADP$$

$$Glucose - 6 - phosphate + NAD^{+} \xrightarrow{G6PDH} Phosphogluconate + NADH$$

3.8.2 Total Cholesterol

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

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

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

$$H_2O_2 + 4 - aminophenazone + Phenol \xrightarrow{peroxidase} H_2\,O + Quinoneimine$$

3.8.3 Triglycerides

The method for this assay is based on a modified Trinder (Barham & Trinder, 1972) colour reaction to produce a fast linear endpoint reaction (McGowan *et al.*, 1983).

Triglycerides in the sample are hydrolyzed by lipase to glycerol and fatty acids. Glycerol is then phosphorylated by adenosine-5-triphosphate (ATP) to glycerol-3phosphate and adenosine-5-diphosphate (ADP) in a reaction catalyzed by glycerol kinase. Glycerol-3-phosphate is then converted to dihydroxyacetone phosphate (DHAP) and hydrogen peroxide (H_2O_2) by glycerophosphate oxidase. The hydrogen peroxide the reacts with 4-aminoantipyrine and 3, 5 dichloro-2-hydroxybenzene (Chlorophenol) in a reaction catalyzed by peroxidase to yield a red coloured

quinoneimine dye. The intensity of the colour produced was directly proportional to the concentration of triglycerides in the sample.

$$Triglyceride + H_2O \xrightarrow{lipase} Glycerol + Fatty\ acids$$

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

$$Glycerol - 3 - phosphate \xrightarrow{glycerolphosphate\ oxidase} DHAP + H_2O_2$$

$$2H_2O_2 + 4 - aminoantipyrine + chlorophenol \xrightarrow{peroxidse} Quinoneimine + 2H_2O_2$$

3.8.4 HDL Cholesterol

Low density lipoproteins (LDL and VLDL) and chylomicron fractions are precipitated quantitatively by the addition of phosphotungstic acid in the presence of Mg2+ ions. The cholesterol concentration in the HDL was then determined by the method described by Trinder for the assay of cholesterol.

3.8.5 LDL Cholesterol

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

$$LDL - Chlesterol\ (mmolL^{-1}) = TC(mmolL^{-1}) - \frac{TG(mmolL^{-1})}{2.2} - HDL(mmolL^{-1})$$

3.8.6 Serum Albumin (ALB)

The method used for this assay was based on that of Doumas *et al.* (1971)where at a controlled pH, bromocresol green (BCG) forms a coloured complex with albumin. The intensity of the colour at 630 nm was directly proportional to the albumin concentration.

$$BCG + Albumin \xrightarrow{controlled\ pH} gREEN\ bcg - Albumin\ Complex$$

3.8.7 Total protein (PRO)

The method was based on the modifications of Gornall *et al.* (1949). Protein in serum forms a blue coloured complex when reacted with cupric ions in an alkaline solution. The intensity of the violet colour was proportional to the concentration of proteins present when compared to a solution with known protein concentration.

$$Protein + Cu^{2+} \xrightarrow{alkali} Coloured\ complex$$

3.8.8 Blood Urea Nitrogen (BUN)

The method for this assay was based on a modification of the Urease/Glutamate dehydrogenase (GLDH) method by Talke and Schubert (1965). Urea is hydrolyzed to ammonia (NH3) and carbon dioxide (CO2) in the presence of water and urease. The liberated ammonia reacts with α-ketoglutarate in the presence of NADH and Glutamate dehydrogenase to form L-Glutamate and NAD+. As the reaction proceeds, the absorbance at 340 nm decreases. The initial rate of this change was proportional to the concentration of urea in the sample.

$$Urea + H_2O \xrightarrow{urease} 2NH_3 + CO_2$$

$$NH_3 + \alpha - Ketoglutarate + NADH \xrightarrow{GLDH} L - Glutamate + NAD^+$$

3.8.9 Creatinine (CRE)

The method for this assay was based on the Jaffe (modified kinetic) method described by Fabiny and Ertingshausen (1971). Creatinine reacts with picric acid in alkaline conditions to form a colour complex which absorbs at 510 nm. The rate of formation of colour was proportional to the concentration of creatinine in the sample.

$$Creatinine + Sodium\ Picrate \xrightarrow{alkali} Creatinine - picrate\ complex$$

3.9 Estimation of Glomerular Filtration Rate

This study assessed renal function in the diabetic patients using the Chronic Kidney

Disease Epidemiology Collaboration (CKD-EPI). Estimated GFR's were used to stratify the study population into the three stages of CKD based on the staging system of the Kidney Disease Outcomes Quality Initiative (K/DOQI) for CKD classification (National Kidney Foundation, 2002) where: Stage 1 (Kidney damage with normal or increased GFR) = GFR ≥ 90 mL min-1 1.73 m^{-2}; Stage 2 (Kidney damage with mildly decreased GFR) = 60-89 mL min-1 1.73 m^{-2}; Stage 3 (Moderately decreased GFR) = 30-59 mL min-1 1.73 m^{-2}; Stage 4 (Severely decreased GFR) = 15–29 mL min-1 1.73 m^{-2} and Stage 5 (Kidney failure) = <15 mL min-1 1.73 m^{-2}(Levey *et al.*, 2005).

3.10 Glycosylated Hemoglobin (HbA1c)

The A_1 fast fraction – cation exchange method was used to estimate the level of glycated hemoglobin of the participants. 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 gave the percentage of glycosylated haemoglobin (GHb).

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

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

3.11 Glycosylated Albumin (GA)

The kit used a double-antibody sandwich enzyme-linked immunosorbent one-step process assay (ELISA) to assay the human glycosylated albumin (GA) level in the samples.

Glycated Albumin (Human) ELISA was a direct non-radiolabel enzyme-linked immunoassay in which glycated albumin in human plasma binds to an immobilized monoclonal antibody that specifically recognizes the glycated moieties on human albumin (Day *et al.*, 1980; Cohen & Hud, 1989). After incubation for a fixed time, an enzyme-conjugated polyclonal antibody directed against human albumin was added. A chromogenic substrate was also added. After the reaction was stopped, the intensity of the color was read in an ELISA reader at 450 nm. The concentration of glycated albumin in the specimen sample was read from a calibration curve.

The amount of glycated albumin can be expressed as absolute concentration (mg/ml) or as a relative %, determined by the equation below;

The percent GA (%) in the sample was then calculated as follows:

$$\%\ \text{Glycated Albumin (GA)} = \frac{\text{Glycated Albumin}_{\text{sample}}}{\text{Total albumin}_{\text{sample}}} X\ 100$$

Where; a) Glycated Albumin is in mg Glycated Albumin /Ml

b) Total Albumin is in mg Albumin /mL

3.12 Diagnostic Criteria for Metabolic Syndrome

Metabolic syndrome in participants were diagnosed using the criteria recommended by the NCEP ATPIII, that is, the presence of three or more of the following risk factors: 1. Central obesity i.e. waist circumference in males >102 cm and females >88 cm, 2.

Hypertriglyceridemia i.e. triglyceride ≥1.70 mmol/L, 3. Low HDL cholesterol i.e. HDL cholesterol in males <1.00 mmol/L and in females <1.30 mmol/L, 4. Hypertension i.e. blood pressure ≥130/85 mmHg and/or on antihypertensive medication, and 5. Hyperglycaemia i.e. a fasting glucose ≥6.1mmol/L. All patients in this study were coded as positive for hyperglycaemia (i.e. glucose ≥6.1mmol/L).

3.13 Glycemic Control

Glycated haemoglobin and glycated albumin were classified as Excellent for HbA1C ≤ 6 and GA ≤ 18; Good for 6 < HbA1C ≤ 7and 18 < GA ≤ 21, Fair for 7 < HbA1C ≤ 8 and 21 < GA ≤ 24; and Poor control as HbA1C > 8 and GA > 24.

3.14 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 Graph Pad 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 Demographics, clinical characteristics and measures of Anthropometry

This case comparative study was conducted at the Diabetic Clinic of the Tema General Hospital where 200 participants were recruited: 150 diabetic patients (participants) and 50 non-diabetics (control).

Table 4.1 presents the baseline characteristics of the participants. The mean ages of the diabetic patients and the non-diabetics were 58.39 ± 12.76 and 54.88 ± 17.90 respectively. Furthermore, majority of the diabetic patients, 28% (42), were in their 6th decade of life however most of the non-diabetics were in their 5thdecade of life, 32.0% (16). Assessments of obesity using Waist circumference and waist-to-hip ratio were significantly (*P<0.0001)* higher in the diabetics than the non-diabetics. BMI comparison among the diabetics and the non-diabetics showed no mean significant differences, however, overweight (38.7%) and obesity (35.3%) was more prevalent in the diabetics. Waist to hip ratio (WHR) was significantly higher in the controls compared to the cases where as the Waist circumference was significantly higher among the cases compared to the controls (Table 4.1).

Table 4.1: Demographics, clinical characteristics and measures of Anthropometry among study participants

Variable	**Cases** (n = 150)	**Controls** (n = 50)	**P-value**
Age (Mean ± SD)	58.39 ± 12.76	54.88 ± 17.90	0.132
Gender n (%)			**< 0.0001**
Male	58 (38.7)	36 (72.0)	
Female	92 (61.3)	14 (28.0)	
Age group in years n (%)			0.230
<30	3 (2.0)	5 (10.0)	
30-39	10 (6.7)	7 (14.0)	

40-49	25 (16.7)	8 (16.0)	
50-59	39 (26.0)	16 (32.0)	
60-69	42 (28.0)	7 (14.0)	
70-79	26 (17.3)	4 (8.0)	
≥ 80	5 (3.3)	3 (6.0)	
Marital status n (%)			**0.001**
Single	27 (18.1)	18 (36.0)	
Married	90 (60.4)	32 (64.0)	
Divorced	4 (2.7)	0 (0.0)	
Widowed	28 (18.8)	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 (%)			0.066
None	21 (14.0)	15 (30.0)	
Basic	62 (41.3)	17 (34.0)	
Secondary	45 (30.0)	14 (28.0)	
Tertiary	22 (14.7)	4 (8.0)	
Sickling Status			0.074
Negative	118 (78.7)	45 (90.0)	
Positive	32 (21.3)	5 (10.0)	
WC (cm)	92.20 ± 12.71	79.54 ± 11.31	**< 0.0001**
WHR	0.90 ± 0.07	0.96 ±0.02	**< 0.0001**
BMI n (Kg/m2)	28.63 ± 5.63	28.71 ± 5.08	0.927
BMI n (%)			0.711
Underweight	5 (3.3)	0 (0.0)	
Normal	34 (22.7)	10 (20.0)	
Overweight	58 (38.7)	22 (44.0)	
Obese	53 (35.3)	18 (36.0)	

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

4.2 Biochemical characteristics, dyslipidemia and measures of renal function

Table 4.2 shows the biochemical characteristics, dyslipidemia and measures of renal function among the diabetic patience and the non-diabetics. Results of blood glucose, Glycated hemoglobin, Glycated albumin, Glycated Albumin/HbA1c and serum albumin were significantly (*P< 0.05)* increased in the patients with diabetes compared to the non-diabetics. However, serum lipid profile although increased in the diabetics, was not statistically different between diabetics and non-diabetics as TC, TG, and LDL

were compared ($P > 0.05$). Renal assessment indicated significant elevations ($P < 0.05$) in levels of urea, creatinine and sodium with increased levels in the diabetic patients. The eGFR was however significantly ($P < 0.0001$) reduced in the diabetics compared to the non-diabetics (Table 4.2)

Table 4 2: Biochemical characteristics, dyslipidemia and measures of renal function among the diabetic patience and the non-diabetics

Variable	Cases (n = 150)	Controls (n = 50)	P-value
FBG (mmol/l)	9.27 ± 3.88	4.78 ± 0.63	**< 0.0001**
HB (g/dl)	11.69 ± 1.69	12.54 ± 1.27	**0.001**
HBA1c (%)	7.43 ± 6.29	6.30 ± 0.95	**< 0.0001**
GA (%)	22.69 ± 4.31	16.33 ± 2.36	**< 0.0001**
GA/HBA1C ratio	3.07 ± 0.28	2.59 ± 0.35	**<0.0001**
Total protein (g/dL)	78.94 ± 7.15	78.12 ± 6.22	0.467
Serum albumin (g/dL)	42.61 ± 4.92	40.07 ± 8.16	**0.009**
Lipid profile TC (mmol/L)	5.10 ± 1.26	4.76 ± 1.17	0.098
TG (mmol/L)	1.20 ± 0.50	1.07 ± 0.58	0.134
HDL-CHL (mmol/L)	1.40 ± 0.45	1.42 ± 0.38	0.711
LDL-CHL (mmol/L)	3.52 ± 1.25	3.15 ± 1.07	0.068
Renal function			
Urea (mmol/L)	4.34 ± 1.20	3.69 ± 1.06	**0.001**
Creatinine (µmol/L)	92.64 ± 20.86	82.85 ± 18.42	**0.003**
Sodium (Na^+) (mmol/L)	137.14 ± 9.25	134.01 ± 10.45	**0.046**
Potassium (K^+) (mmol/L)	4.04 ±0.63	4.10 ± 0.37	0.506
eGFR, mL/min/1.73 m²	79.96 ± 25.80	112.84 ± 26.25	**< 0.0001**

FBG=Fasting Blood Glucose, HB=Haemoglobin, HbA1C=Glycated haemoglobin, GA=Glycated Albumin, 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

Presented in table 4.3 is age distribution, measure of Anthropometry, and prevalence of disease complication among diabetic patients. The mean ages of the diabetic male and female patients were 57.82 ± 14.49 and 58.74 ± 11.61 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). BMI was however significantly different

between genders, with overweight (58.6%) and obesity (75.5%) being more prevalent in the females than the male diabetics. Diabetic nephropathy and retinopathy were also more prevalent in the female diabetics (68.3%, 63.3% respectively) than the male diabetics (31.7%, 36.7% respectively) (Table 4.3).

Table 4.3: Age distribution, measure of Anthropometry, and prevalence of disease complication among the diabetic patients stratified by gender

Variable	Male (n = 58)	Female (n = 92)	P-value
Age (Mean ± SD)	57.82 ± 14.49	58.74 ± 11.61	0.672
Age group n (%)			0.347
<30	3 (100)	0 (0.0)	
30-39	4 (40.0)	6 (60.0)	
40-49	8 (32.0)	17 (68.0)	
50-59	12 (30.8)	27 (69.2)	
60-69	18 (42.9)	24 (57.1)	
70-79	11 (42.3)	15 (57.7)	
≥ 80	2 (40.0)	3 (60.0)	
Sickling Status			0.331
Negative	48 (40.7)	70 (59.3)	
Positive	10 (31.2)	22 (68.8)	
WC (cm)	90.77 ± 11.70	93.10 ± 13.29	0.277
WHR	0.90 ± 0.07	0.90 ±0.07	0.688
BMI n (Kg/m2)	27.10 ± 5.12	29.59 ± 5.75	**0.008**
BMI n (%)			**0.015**
Underweight	1 (33.3)	2 (66.7)	
Normal	20 (58.8)	14 (41.2)	
Overweight	24 (41.4)	34 (58.6)	
Obese	13 (24.5)	40 (75.5)	
Disease complication			0.467
None	34 (43.0)	45 (57.0)	
Nephropathy	13 (31.7)	28 (68.3)	
Retinopathy	11 (36.7)	19 (63.3)	

4.4 Glycemic indices, dyslipidemia and measures of renal function among the diabetic patients

Table 4.4 shows Glycemic indices, dyslipidemia and measures of renal function among the diabetic patients. Glycated haemoglobin and serum albumin were not significantly (*P*>0.05*)* different between the male and female diabetics (Table 4.4). However, Glycated albumin was significantly increased in the female diabetics. Serum lipid profile although increased in the female diabetics (with the exception of TG), showed no statistically significant difference between the genders as TC, TG, HDL and LDL

were compared (P> 0.05). Renal assessment indicated no significant difference (P> 0.05) in levels of urea, creatinine, sodium and potassium. However, the estimated GFR was significantly (P<0.0001) reduced in the females compared to the male diabetics (Table 4.4)

Table 4.4: Glycemic indices, dyslipidemia and measures of renal function among the diabetic patients in relation to Gender

Variable	Male (n = 58)	Female (n = 92)	P-value
FBG (mmol/l)	9.28 ± 4.55	9.26 ± 3.43	0.974
HB (g/dl)	11.98 ± 2.11	11.50 ± 1.33	0.089
HBA1c (%)	7.16 ± 1.36	7.60 ± 1.47	0.069
GA (%)	21.68 ± 4.32	23.34 ± 4.20	**0.021**
GA/HBA1C ratio	3.03 ± 0.26	3.09 ± 0.29	0.200
Total protein (g/dL)	79.13 ± 7.52	78.82 ± 6.94	0.795
Serum albumin (g/dL)	42.34 ± 5.96	42.79 ± 4.16	0.592
Lipid profile TC (mmol/L)	4.93 ± 1.20	5.21 ± 1.30	0.191
TG (mmol/L)	1.23 ± 0.66	1.17 ± 0.36	0.476
HDL-CHL (mmol/L)	1.32 ± 0.45	1.45 ± 0.44	0.074
LDL-CHL (mmol/L)	3.42 ± 1.21	3.58 ± 1.28	0.453
Renal fuction Urea (mmol/L)	4.31 ± 1.32	4.36 ± 1.13	0.778
Creatinine (µmol/L)	94.79 ± 22.66	91.28 ± 19.66	0.318
Sodium (Na+) (mmol/L)	137.49 ± 8.09	136.92 ± 9.94	0.71
Potassium (K+) (mmol/L)	4.07 ± 0.75	4.01 ± 0.54	0.594
eGFR, mL/min/1.73 m^2	91.67 ± 26.24	72.58 ± 22.72	**< 0.0001**

4.5 Proportion of glycemic control and Disease complications

Table 4.5 shows the proportion of glycemic control among diabetic patients. Glycemic control was assessed by estimating Glycated haemoglobin (HbA1c) and Glycated albumin (GA) (Table 4.5). The proportion of excellent control of blood glucose assessed using GA, 11.3%, was lower than that assessed by HbA1c (16.7%). Also, glycemic control assessed by GA showed a greater proportion of poor control (35.3%) than when assessed by HbA1c (28.7%) (Table 4.5).

Across the various age groups as shown in fig 4.1, diabetic nephropathy (29.3%) was more prevalent in the diabetic patients aged between 70-79 years and retinopathy (43.3%) more prevalent in the patients aged 60-69 years. The patients aged < 30 years did not present with diabetic nephropathy, however 3.3 % of them presented with diabetic retinopathy (Figure 4.1).

Table 4.5: Proportion of glycemic control among diabetic patients assessed by HbA1c and GA

Glycemic Control	HbA1c (%)	GA (%)
Excellent (HbA1C ≤ 6, GA ≤ 18)	25 (16.7)	17 (11.3)
Good (6 < HbA1C ≤ 7, 18 < GA ≤ 21)	43 (28.7)	38 (25.3)
Fair (7 < HbA1C ≤ 8, 21 < GA ≤ 24)	39 (26.0)	42 (28.0)
Poor (HbA1C > 8, GA > 24)	43 (28.7)	53 (35.3)

Numbers in parentheses indicate the percentage of whole patients (n = 150)

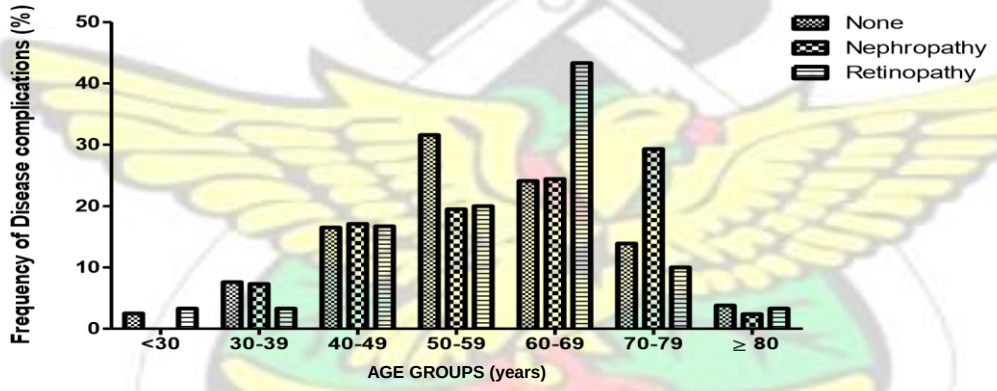


Figure 4.1: Percentage occurrence of complications of diabetes across the various age distributions

4.6 Anthropometry, dyslipidemia and renal function among the diabetic patients in relation to their sickling status

Table 4.6 presents Anthropometry, dyslipidemia and renal function among the diabetic patients in relation to their sickling status. The diabetic patients with SCD showed no statistically significant difference as WC, WHR, BMI and serum lipid profile parameters were compared with those without SCD, except for HDL levels (P= 0.034). Renal function parameters although increased in the diabetic SCD patients showed no

significant difference when compared to diabetics with no SCD. Estimated GFR was lower (P=0.401) in those with SCD (Table 4.6).

Table 4.6: Anthropometry, dyslipidemia and renal function among the diabetic patients in relation to their sickling status Variable Sickling status P-value

	Positive (n = 32)	**Negative** (n = 118)	
WC (cm)	92.72 ± 10.66	92.06 ± 13.25	0.797
WHR	0.89 ± 0.07	0.90 ± 0.07	0.468
BMI n (Kg/m2)	29.21 ± 4.82	28.47 ± 5.84	0.510
BMI n (%)			0.261
Underweight	0 (0.0)	3 (2.5)	
Normal	5 (15.6)	30 (25.4)	
Overweight	17 (53.1)	42 (35.6)	
Obese	10 (31.2)	43 (36.4)	
Lipid profile TC (mmol/L)	5.28 ± 1.12	5.05 ± 1.30	0.377
TG (mmol/L)	1.23 ± 0.34	1.19 ± 0.53	0.687
HDL-CHL (mmol/L)	1.25 ± 0.42	1.44 ± 0.45	**0.034**
LDL-CHL (mmol/L)	3.79 ± 1.15	3.44 ± 1.27	0.158
Renal function			
Urea (mmol/L)	4.54 ± 1.41	4.29 ± 1.14	0.286
Creatinine (µmol/L)	93.44 ± 17.11	92.42 ± 21.83	0.807
Sodium (Na+) (mmol/L)	137.09 ± 7.00	137.15 ± 9.79	0.975
Potassium (K+) (mmol/L)	4.15 ± 0.83	4.01 ± 0.57	0.263
eGFR, mL/min/1.73 m^2	76.55 ± 21.30	80.89 ± 26.90	0.401
eGFR, n (%)			0.961
< 60	8 (25.0)	30 (25.4)	
≥ 60	24 (75.0)	88 (74.6)	

4.7 Glycemic indices and proportion of glycemic control among the diabetic patients stratified by their sickling status

Table 4.7 from the study shows that glycemic indices among the diabetics with SCD and those without SCD showed no statistically significant difference (P>0.05) as presented in Table 4.7. Among the diabetics with SCD, 46.9% and 93.8% poorly controlled their blood glucose assessed by HbA1c and GA respectively with 25.0% developing nephropathy and 21.9% retinopathy (Table 4.7).

Table 4.7: Glycaemic indices and proportion of glycemic control among the diabetic patients stratified by their sickling status

Variable	Sickling status		P-value
	Positive (n= 32)	Negative (n= 118)	
HB (g/dl)	11.18 ± 1.62	11.83 ± 1.69	0.056
Total protein (g/dL)	79.66 ± 6.13	78.75 ± 7.41	0.523
Serum albumin (g/dL)	42.17 ± 3.75	42.73 ± 5.20	0.566
Glycemic Indices FBG (mmol/L)	9.58 ± 3.68	9.18 ± 3.95	0.610
HbA1c (%)	6.99 ± 1.10	7.55 ± 1.50	0.051
GA (%)	21.86 ± 3.62	22.93 ± 4.47	0.217
Glycemic Control (HbA1c)			0.318
> 7 (poor control)	15 (46.9)	67 (56.8)	
≤ 7 (Good control)	17 (53.1)	51 (43.2)	
Glycemic Control (GA)			0.762
> 21 (poor control)	21 (65.6)	74 (62.7)	
≤ 21 (Good control)	11 (34.4)	44 (37.3)	
Disease complication			0.926
None	17 (53.1)	62 (52.5)	
Nephropathy	8 (25.0)	33 (28.0)	
Retinopathy	7 (21.9)	23 (19.5)	

4.8 Glycemic indices and proportion of glycaemic control of patients with diabetes stratified by the disease complication

Table 4.8 describes Glycemic indices and proportion of glycaemic control of patients with diabetes stratified by the disease complication. In the patients with diabetic nephropathy, HbA1c and GA were significantly ($P< 0.05$) lower compared to those with retinopathy. ANOVA multiple comparisons of the various complications showed significant differences in levels of total protein and serum albumin ($P<0.0001$, $P =0.008$ respectively). However, the difference in the levels of total protein and serum albumin among the patients with retinopathy and those without any complication were not significant (Table 4.8). In the patients with nephropathy, 17.1% and 14.6% excellently controlled their blood glucose whilst 14.6% and 17.1% poorly did as assessed by

HbA1c and GA respectively. On the other hand, 20.0% of the patients with retinopathy excellently controlled their blood glucose whilst 36.7% and 50.0%did poorly (HbA1c and GA respectively).

Table 4.8: Glycemic indices and proportion of glycemic control of patients with diabetes stratified by the disease complication Variables Disease

Complications (n = 41)	P-value	None (n = 79)	Nephropathy	Retinopathy (n = 30)	
HB (g/dl)	11.18 ± 1.06^{*+}	11.82 ± 1.62*		12.05 ± 2.34^{+}	0.061
Total protein (g/dL)		80.93 ± 5.54*	74.46 ± 9.36^{*+}	79.84 ± 4.38^{+}	**< 0.0001**
Serum albumin (g/dL)		42.94 ± 4.61*	40.78 ± 5.96^{*+}	44.25 ± 3.23^{+}	**0.008**
Glycemic Indices					
FBG (mmol/L)		9.21 ± 3.64	9.39 ± 4.12	9.24 ± 4.29	0.970
HbA1c (%)		7.59 ± 1.45*	6.90 ± 0.97^{*+}	7.73 ± 1.77^{+}	**0.018**
GA (%)		23.29 ± 4.33	20.74 ± 2.99*$^{+}$	23.83 ± 4.99^{+}	**0.002**
GA/HbA1c ratio		3.08 ± 0.26	3.01 ± 0.28	3.10 ± 0.33	0.344
HbA1c n (%)					0.162
Excellent		12 (15.2)	7 (17.1)	6 (20.0)	
Good		19 (24.1)	18 (43.9)	6 (20.0)	
Fair		22 (27.8)	10 (24.4)	7 (23.3)	
Poor		26 (32.9)	6 (14.6)	11 (36.7)	
GA n (%)					**0.005**
Excellent		5 (6.3)	6 (14.6)	6 (20.0)	
Good		22 (27.8)	15 (36.6)	1 (3.3)	
Fair		21 (26.6)	13 (31.7)	8 (26.7)	
Poor		31 (39.2)	7 (17.1)	15 (50.0)	

****significantly different on comparison to None group, $^{+}$ significantly different on comparison to Nephropathy group at P < 0.05, Excellent (HbA1c ≤ 6, GA ≤ 18), Good (6 < HbA1c ≤ 7, 18 < GA ≤ 21), Fair (7 < HbA1c ≤ 8, 21 < GA ≤ 24), Poor (HbA1c > 8, GA > 24)***

4.9 Anthropometry, dyslipidemia and renal function of the diabetic patients with and without disease complications

Obesity, dyslipidemia and renal function of the diabetic patients with and without disease complications is presented in table 4.9. Waist circumference, waist-to-hip ratio and BMI showed no statistically significant difference on comparison across the disease complication (Table 4.9). Overweight was observed in 48.8% and 36.7% of the patients with nephropathy and retinopathy respectively. On the other hand, obesity was 26.8% and 40.0% 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, and LDL were compared ($P > 0.05$) except for HDL (P=0.019) (Table 4.9).Renal assessment indicated no significant difference ($P > 0.05$) in levels of urea, creatinine, and potassium. However, sodium was significantly different on comparison ($P < 0.045$).

Table 4.9: Anthropometry, dyslipidemia and renal function of the diabetic patients with and without disease complications Variables Disease Complication

P-value				
	None	**Nephropathy**	**Retinopathy**	
WC (cm)	92.11 ± 14.51	90.15 ± 10.38	95.23 ± 9.97	0.250
WHR	0.90 ± 0.07	0.89 ± 0.08^{+}	0.92 ± 0.05^{+}	0.135
BMI n (Kg/m2)	28.79 ± 6.03	28.21 ± 5.10	28.77 ± 5.38	0.857
BMI n (%)				0.678
Underweight	2 (2.6)	0 (0.0)	1 (3.3)	
Normal	18 (23.4)	10 (24.4)	6 (20.0)	
Overweight	27 (35.1)	20 (48.8)	11 (36.7)	
Obese	30 (39.0)	11 (26.8)	12 (40.0)	
Lipid profile TC (mmol/L)	5.04 ± 1.20	5.07 ± 1.46	5.29 ± 1.15	0.627
TG (mmol/L)	1.22 ± 0.59	1.13 ± 0.36	1.22 ± 0.38	0.619
HDL-CHL (mmol/L)	1.40 ± 0.47	1.27 ± 0.42^{+}	1.57 ± 0.37^{+}	**0.019**
LDL-CHL (mmol/L)	3.44 ± 1.21	3.66 ± 1.49	3.51 ± 1.02	0.668

Renal fuction				
Urea (mmol/L)	4.35 ± 1.07	4.21 ± 1.63	4.50 ± 0.83	0.601
Creatinine (µmol/L)	93.85 ± 18.52	87.46 ± 25.49	96.53 ± 18.98	0.147
Sodium (Na+) (mmol/L)	137.89 ± 7.97*	134.21 ± 13.14*+	139.17 ± 3.53+	**0.047**
Potassium (K+) (mmol/L)	3.96 ± 0.35	4.18 ± 1.03	4.03 ± 0.47	0.193

significantly different on comparison to None group, + significantly different on comparison to Nephropathy group at P < 0.05

4.10 Renal impairment assessed by estimated glomerular filtration rate (eGFR) among diabetic patients in relation to their disease complications

Table 4.9 shows renal impairment assessed by estimated glomerular filtration rate (eGFR) among diabetic patients. Estimated glomerular filtration rate was reduced in the patients with retinopathy compared to the other groups where 33.3% had eGFR< 60 *mL/min/1.73 m²*. Furthermore, 24.4% of the patients with retinopathy and 22.8% of those without any disease complication had eGFR< 60 *mL/min/1.73 m²*. Moderately reduced renal function (eGFR, 30-59 *mL/min/1.73 m²*) was observed in 22.9% of patients without any complications, 24.4% of those with nephropathy and 33.0% with retinopathy (Table 4.10)

Table 4.10: Renal impairment assessed by estimated glomerular filtration rate (eGFR) among diabetic patients in relation to their disease complications

Variable	**Disease Complication**			**P-value**
	None	**Nephropathy**	**Retinopathy**	
eGFR*, *mL/min/1.73 m²	80.02 ± 25.31	83.86 ± 58.51	74.48 ± 22.87	0.32
eGFR, n (%)				0.521
< 60	18 (22.8)	10 (24.4)	10 (33.3)	
≥ 60	61 (77.2)	31 (75.6)	20 (66.7)	
Renal state: eGFR, n (%)				0.272
Stage 1: ≥ 90	22 (27.8)	17 (41.5)	8 (26.7)	
Stage 2: 60-89	39 (49.4)	14 (34.1)	12 (40.0)	
Stage 3a: 45-59	17 (21.6)	8 (19.5)	10 (33.3)	
Stage 3b:30-44	1 (1.3)	2 (4.9)	0 (0.0)	

4.11 Clinical characteristics of the patients with metabolic syndrome

Table 4.11 presents the clinical characteristics of the patients with metabolic syndrome. Metabolic syndrome was observed in 4.7% of the patients with diabetes. Total protein, serum albumin, HbA1c and GA were observed to be increased in the patients with metabolic syndrome however the differences were not statistically significant (P> 0.05). A greater proportion (85.7%) of those with metabolic syndrome had poor glycaemic control as assessed by both criteria. Diabetic nephropathy and retinopathy were present in 28.6% and 14.3% of the patients with metabolic syndrome respectively. **Table 4.11: Clinical characteristics of the patients with metabolic syndrome**

Variable	Metabolic Syndrome		P-value
	YES (n =7)	**NO** (n=143)	
HB (g/dl)	12.84 ± 0.59	11.63 ± 1.70	0.064
Total protein (g/dL)	79.11 ± 3.71	78.93 ± 7.28	0.948
Serum albumin (g/dL)	44.24 ± 5.00	42.53 ± 4.92	0.371
Glycemic Indices			
HbA1c (%)	7.74 ± 1.16	7.41 ± 1.45	0.560
GA (%)	24.53 ± 4.76	22.61 ± 4.29	0.251
Glycemic Control (HbA1c)			0.091
> 7 (poor control)	6 (85.7)	76 (53.1)	
≤ 7 (Good control)	1 (14.3)	67 (46.9)	
Glycemic Control (GA)			0.208
> 21 (poor control)	6 (85.7)	89 (62.2)	
≤ 21 (Good control)	1 (14.3)	54 (37.8)	
Renal function			
Urea (mmol/L)	4.44 ± 1.08	4.34 ± 1.21	0.822
Creatinine (mmol/L)	82.51 ± 26.81	93.14 ± 20.52	0.189
Sodium (Na+) (mmol/L)	139.31 ± 4.52	137.03 ± 9.41	0.526
Potassium (K+) (mmol/L)	3.83 ± 0.52	4.05 ± 0.64	0.390

eGFR, mL/min/1.73 m²	93.91 ± 24.79	79.28 ± 25.74	0.143
Disease complication			0.927
None	4 (57.1)	75 (52.4)	
Nephropathy	2 (28.6)	39 (27.3)	
Retinopathy	1 (14.3)	29 (20.3)	

Fig. 4.2 & 4.3 Correlation analysis of HbA1c, GA, HB and Serum albumin

In figure 4.2 below, a highly significant relationship (P<0.001) was established between the levels of HbA1c and Glycated albumin among patients with diabetes (A) and those without diabetes (B).

The relationship between HbA1c and haemoglobin showed a positive correlation in both diabetics and non-diabetics although was not significant (P>0.05). On the other hand, a significantly direct association was observed between Glycated haemoglobin and serum albumin levels in diabetic patients (r=0.264, P=0.001). However, the relationship observed in the non-diabetics was no statistically significant (r= 0.186, P=0.195) (Figure 4.3).

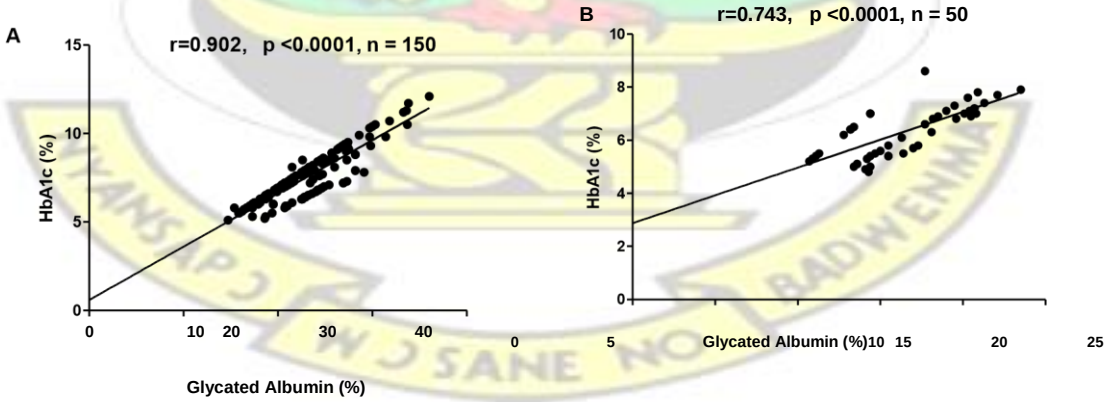
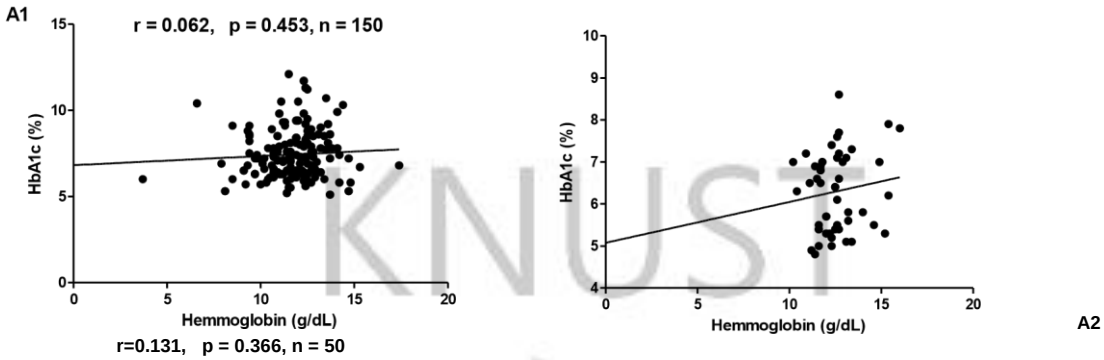


Figure 4.2: Relationship between HbA1c and GA levels in patients with diabetes (A) and without diabetes (B), r= Pearson's correlation coefficient, n = Number of patients



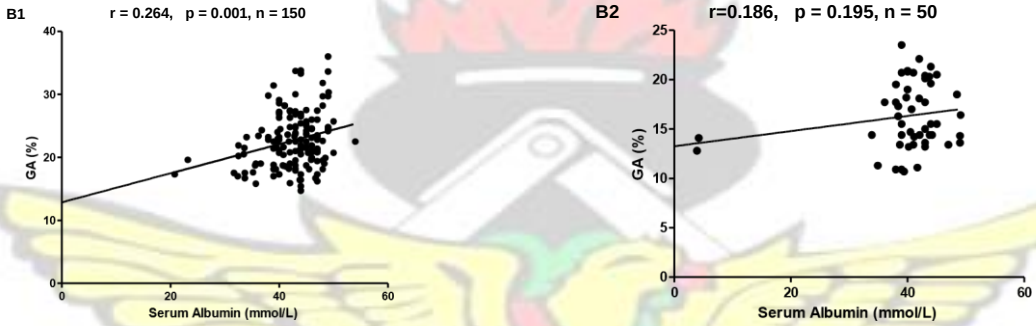


Figure 4.3: Relationship between HbA1c and Haemoglobin; and GA and Serum albumin levels in patients with diabetes (A1, B1) and without diabetes (A2, B2), r= Pearson's correlation coefficient, n = Number of patients

4.12 Relationship between glycemic indices, age, measures of obesity, total protein and serum albumin

The relationship between glycemic indices, age, measures of obesity, total protein and serum albumin is presented in table 4.11. Glycated haemoglobin significantly and directly correlates with Glycated albumin, total protein and serum albumin ($P<0.05$) among the diabetic patients, whereas it correlates inversely with Age (r= -0.015, P=0.859) and BMI (r= - 0.017, P=0.709).

Glycated albumin on the other hand is positively and significantly associated with total protein (r= 0.197, P= 0.016) and serum albumin (r= 0.264, P= 0.001). Age and WHR were negatively correlated and showed no significance (Table 4.11).

Table 4.12: Relationship between glycemic indices, age, measures of obesity, total protein and serum albumin (Person's correlation)

Parameters		HbA1c	GA	AGE	BMI	WHR	HB	TP	S.ALB
HbA1c	R	1	0.902**	-0.015	-0.017	0.031	0.062	0.215**	0.262**
	P-value		0.000	0.859	0.841	0.709	0.453	0.008	0.001
GA	R		1	-0.057	0.069	-0.003	0.087	.197*	.264**
	P-value			0.486	0.401	0.969	0.292	0.016	0.001
AGE	R			1	-0.072	-0.106	0.053	-0.069	-0.054
	P-value				0.382	0.198	0.52	0.404	0.509
BMI	R				1	0.113	-0.057	0.01	-0.01
	P-value					0.170	0.486	0.902	0.902
WHR	R					1	0.204*	0.139	0.216**
	P-value						0.012	0.091	0.008
HB	R						1	0.091	0.135
	P-value							0.266	0.099
TP	R							1	0.528**
	P-value								0.000
S. ALB.	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 Relationship between glycemic indices and dyslipidemia

Table 4.12 shows the relationship between glycemic indices and dyslipidemia. Glycated haemoglobin and glycated albumin both showed an inverse but insignificant relationship with total cholesterol (TC) levels (r= -0.073, P= 0.377; r= -0.041, P=0.617 respectively) and LDL (r= -0.083, P= 0.311; r= -0.090, P=0.272 respectively). However a direct relationship with TG, HDL with no significance (P>0.05) was observed (Table 4.12)

Table 4.13: Relationship between glycemic indices, age, measures of obesity, total protein and serum albumin (Person's correlation)

Parameters		HbA1c	GA	FBS	TC	TG	HDL	LDL
HbA1c	R	1	0.902**	0.049	-0.073	0.014	0.055	-0.083
	P-value		0.000	0.550	0.377	0.863	0.502	0.311
GA	R		1	-0.006	-0.041	0.002	0.078	-0.09
	P-value			0.943	0.617	0.977	0.343	0.272
FBG	R			1	-0.025	-0.061	0.107	-0.012
	P-value				0.764	0.460	0.193	0.886
TC	R				1	0.161*	0.208*	0.882**
	P-value					0.049	0.011	0.000
TG	R					1	-0.199*	0.112
	P-value						0.015	0.172
HDL	R						1	-0.069
	P-value							0.403
LDL	R							1
	P-value							

4.14 Relationship between glycemic indices and measures of renal function

The relationship between glycemic indices and measures of renal function is shown in table 4.14. The glycemic indices showed inverse correlation with potassium levels and estimated GFR. Urea, creatinine and sodium on the other hand were directly but not statistically significantly associated with the glycemic indices (Table 4.13)

Table 4.14: Relationship between glycemic indices and measures of renal function (Person's correlation)

Parameters		HbA1c	GA	Urea	Creatinine	Sodium (Na^+)	Potassium (K^+)	eGFR
HbA1c	R	1	0.902**	0.031	0.073	0.075	-0.123	-0.125
	P-value		0.000	0.704	0.373	0.359	0.134	0.127
GA	R		1	0.066	0.017	0.092	-0.151	-0.098
	P-value			0.426	0.836	0.263	0.065	0.234
Urea	R			1	0.588**	0.075	0.165*	-0.445**
	P-value				0.000	0.361	0.044	0.000
Creatinine	R				1	0.242**	0.022	-0.820**

	P-value	0.003	0.785	0.000
Sodium (Na^+)	R	1	-0.243**	-0.232**
	P-value		0.003	0.004
Potassium (K^+)	R		1	0.047
	P-value			0.571
eGFR	R			1
	P-value			

4.15 Determinants of poor glycaemic control among participants

Table 4.15 shows the Logistic regression of determinants for poor glycaemic control. The risk of having poor glycaemic control assessed by GA (OR=3.25, P=0.001) was significantly higher compared to that assessed by HbA1c (OR= 1.52, P=0.213) for female diabetics. Diabetic patients with complications showed minimal association with poor glycaemic control as assessed by HbA1c. However, diabetics with retinopathy showed high risk of poor control assessed by GA (OR=1.71, P=0.278). Poor glycaemic control determined by HbA1c and GA were highly associated with Obesity (Table 4.14). Reduced kidney function was determined to be high risk factor in developing poor glycemic control assessed by both criteria.

Table 4.15: Logistic regression of determinants for poor glycaemic control assessed by HbA1c and Glycated Albumin (GA) in diabetic patients

Variable	**HbA1c**		**GA**	
	OR (95% CI)	**P-value**	**OR (95% CI)**	**P-value**
Gender Male*				
	Reference		Reference	
Female	1.52 (0.79-2.95)	0.213	3.25 (1.62-6.52)	0.001
Disease Condition				
None*	Reference		Reference	
Nephropathy	0.41 (0.19-0.90)	0.025	0.50 (0.23-1.07)	0.073
Retinopathy	0.97 (0.41-2.29)	0.942	1.71 (0.65-4.48)	0.278
BMI				
Underweight	1.50 (0.12-18.13)	0.750	0.92 (0.08-11.22)	0.946

Normal*	Reference		Reference	
Overweight	0.59 (0.25-1.37)	0.222	0.44 (0.18-1.07)	0.069
Obese	1.34 (0.56-3.22)	0.509	1.41 (0.55-3.64)	0.478
eGFR,				
≥ 60	Reference		Reference	
< 60	1.60 (0.75-3.40)	0.226	1.35 (0.62-2.95)	0.452

OR=Odds Ratio, CI= Confidence Interval

4.16 Area under the curve for HbA1c and GA as markers for Glycemic control

Figure 4.4 shows the receiver operator curve for HbA1c and GA as markers for Glycemic control. The area under the ROC curve for HbA1c and GA was 0.732 and 0.879 respectively suggesting that both markers for glycemic control are good but, GA is a better maker with higher AUC.

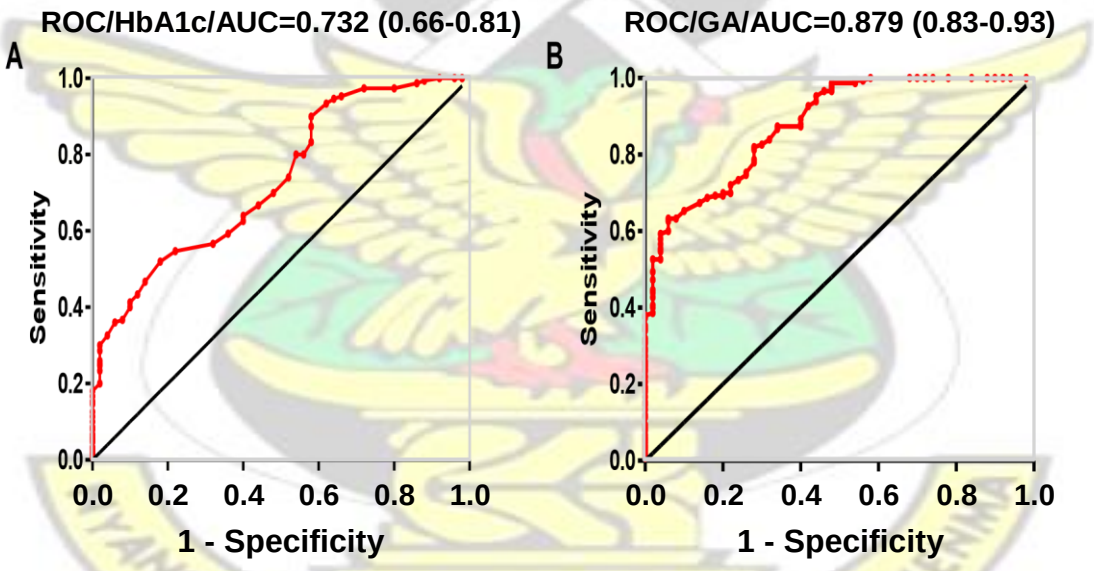


Figure 4 4 Receiver Operator Curve and Area under the curve (AUC) for HbA1c (A) and GA (B) in the management of T2DM

4.17 Accuracy of Glycemic control in the management of T2DM

Table 4.16 shows the Accuracy of HbA1c and GA as Glycemic control in the management of T2DM. The diagnostic value of 7.15% for HbA1c had a sensitivity of

52.0% and Specificity of 82.0% for Glycemic control in the management of T2DM and 20.60% diagnostic value with 67.3% sensitivity and 86.0% Specificity for GA (Table 4.16).

Table 4.16 Accuracy of Glycemic control in the management of T2DM

Threshold values	Sensitivity (95% CI)	Specificity (95% CI)	AUC (95% CI)	P-value
T2DM				
HbA1c (%)				
7.15	52.0% (43.7-60.2)	82.0% (68.6-91.4)	0.732 (0.66-0.81)	<0.0001
GA (%)				
20.60	67.3% (59.2-74.8)	86.0% (73.3-94.2)	0.879 (0.83-0.93)	<0.0001

CHAPTER 5

DISCUSSION

5.1 Introduction

This case comparative study was conducted at the Diabetic Clinic of the Tema General Hospital with a total of 200 participants recruited for the study of which 150 were known and confirmed diabetics and 50 were healthy individuals attending the Hospital. The 150 diabetics consisted of 79 diabetics without any complications, 41 with diabetic renal damage and 30 with diabetic retinopathy. According to the International Diabetes Federation (Atlas, 2009) the peak age for onset of diabetes in 2010 is 40–59 years, but by 2030, the highest prevalence will be in the oldest age-group (60–79 years). This was also reflected in this study where the mean age of the diabetics was 58.39 ±12.76 years. The high proportion of females (61.3%) in this study may be due to the nature of the population being admitted to this hospital in that more women 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 (28.0%) of diabetic

patients in this study was found in the age group, 60 – 69 years. However, a sizeable percentage (20.6%), were ≥70 years. Only a low percentage (8.7%) 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 on diabetes prevalence is already evident (Elbagir *et al.*, 1996; Christensen *et al.*, 2009).

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; furthermore the majority of people with diabetes mellitus in developing countries 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 and 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 mean waist circumference and WHR were observed between diabetic patients and nondiabetics. Mean BMI was not significantly different between diabetic patients and nondiabetics

however overweight (39.2%) and obesity (35.8%) 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). Researchers from Southern 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 (27.10 ± 5.12 kg/m^2) and females (29.59 ± 5.75 kg/m^2) with diabetes both indicate overweight, however, the females have a significantly higher BMI (P = 0.008) than males. Similarly, the percentage of female diabetics who were obese (75.5%) and overweight (58.6%) were significantly higher than the corresponding values of 24.5% and 41.4% for male diabetics (P = 0.015). 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 oestrogen as it reduces their ability to burn energy after eating which results in increased 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 and BMI in the diabetic females in this study. Thus, there is a higher

percentage of Ghanaian female diabetics (61.3%) than males (38.7%) whose condition may be associated with obesity in the current study. 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 Dyslipidemia (Total cholesterol, Triglyceride, LDL, HDL) in T2DM

In diabetes many factors may affect blood lipid levels, because of interrelationship between carbohydrate and lipid metabolism. Therefore, any disorder in carbohydrate metabolism leads to disorder in lipid metabolism and vice versa. Insulin resistance is a primary defect in the majority of patients with T2DM (Haffner *et al.*, 2000). 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. 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: dyslipidemia, platelet activation, and altered endothelial metabolism (Jokl & Colwell, 1997; Brownlee, 2001; Taskinen, 2003). 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 observed in patients with diabetes, which are well known risk factors for cardiovascular diseases among patients, when compared to the patients with no diabetes

(Table 4.2). 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 Renal function in T2DM

There were significant increases in urea and creatinine among diabetics than the nondiabetics in this study. Increased blood urea concentration with increasing blood sugar levels demonstrated in this clearly illustrates the association between hyperglycaemia and damage to the kidney (Zimmet *et al.*, 2001; Shrestha *et al.*, 2008). This finding corroborates the findings of Shrestha *et al.* (2008) that hyperglycemia is one of the major causes of progressive renal damage. Furthermore, Adler *et al.* (2003) indicated that raised plasma creatinine and urea levels among diabetic patients may indicate a pre-renal problem such as volume depletion. Judykay (2007) also proposed that high creatinine levels observed in diabetic patients may be due to impaired function of the nephrons.

As expected the male diabetics showed slightly higher creatinine levels than the females but the differences were not statistically significant. This finding is consistent with established knowledge that blood creatinine levels are influenced by gender. The high serum creatinine levels seen in males compared to females is attributable to the presence of high muscle mass in males (Anjaneyulu & Chopra, 2004; Ashavaid *et al.*, 2005;

Singh *et al.*, 2014). Anjaneyulu *et al.* (2004) confirmed in their studies that increasing serum urea and serum creatinine among diabetics indicates progressive renal damage.

Patients with type 2 diabetes have an increased risk for cardiovascular and chronic kidney disease. Superimposed hypertension further increases the risk and is associated with increased dietary sodium intake(Provenzano *et al.*, 2014). This may account for the significant increase in sodium levels established among the diabetic patients.

In addition, eGFR was significantly reduced ($P<0.0001$) among the diabetics compared to the non-diabetics indicating probable deterioration of renal function in the diabetics.

According to the guidelines of the National Kidney Foundation for the diagnosis and stratification of chronic kidney diseases, renal function is moderately decreased if GFR is <60 mL/min/1.73 m^2 and severely decreased if GFR is <30 mL/min/1.73 m^2 (Levey *et al.*, 2003). The study has shown that 25.3% of the diabetics had GFR <60 mL/min/1.73 m^2. Other studies have also reported decreased renal function among patients with diabetes (Dukas *et al.*, 2005; Kengne *et al.*, 2005). This study also established that about 25.0% of the diabetic SCD patients presented with moderately decreased renal function. This subset of diabetics with SCD who have low eGFR, hypertension and/or albuminuria may be at particular risk for development of overt sickle cell nephropathy and/or advanced CKD and merit close attention (Lu *et al.*, 2011).

5.5 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, as well as poor management by health care professionals (Fitzgerald *et al.*, 1995; Ajayi & Ajayi, 2009; Sharma *et al.*, 2011). The

high prevalence of nephropathy (27.3%) followed by retinopathy (20.0%), which are the most specific complications 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 (43.3%) 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%, 31.7% nephropathy in the females and males respectively and 63.3%, 36.7% 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).

5.6 Variations in Glycated hemoglobin, Glycated albumin and Glycemic control assessed by both criteria in T2DM

This study showed significantly (P=0.001) increased glycated haemoglobin, Glycated albumin levels and high GA-HbA1c ratio in type-2 diabetics compared with nondiabetics. These findings were in accordance to the study of (Koga *et al.*, 2007; 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 nonenzymatic glycation of haemoglobin (Chen *et al.*, 1996; Ahmad, 2005) hence the increase in HbA1c observed in this study.

The glycation efficiency depends on the nature and the anomerization of the carbohydrate involved in the process. In vivo studies demonstrated that the proportion of glycated albumin in healthy persons is in the range of 1- 10% , compared with diabetic individuals in whom this may increase two- to three fold (Bourdon *et al.*, 1999).

Poor glycemic control was generally increased using both criteria; however, poor control assessed by Glycated albumin was higher (63.3%). As serum GA reflects shorter terms of glycemic control than HbA1c, GA changes more rapidly than HbA1c as glycemic control changes (Tahara & Shima, 1995; Koga *et al.*, 2011; Won *et al.*, 2012). GA is indicated to reflect plasma glucose excursions and/or postprandial glucose levels better than HbA1c (Yoshiuchi *et al.*, 2008; Sakuma *et al.*, 2011). Previous study show that endogenous insulin secretion had inverse correlation with the GA/HbA1c ratio in patients with type 2 diabetes mellitus (Koga *et al.*, 2010), suggesting that in diabetic patients with decreased insulin secretion, GA levels are set higher relative to HbA1c because of marked plasma glucose excursions. The GA/HbA1c ratio also decreases as glycemic control improves and increases as glycemic control worsens

(Takahashi *et al.*, 2007; Murai *et al.*, 2013). In the present study, higher GA/HbA1c ratio was also observed in the diabetics.

In accordance with the study of Morita *et al.* (2013) mean levels of GA and the GA/HbA1c ratio were significantly higher in patients with diabetic retinopathy than in patients without diabetic retinopathy. Taken together with these observations, patients with higher postprandial glucose levels are prone to show higher levels of GA in relation to HbA1c and to develop diabetic retinopathy. In previous studies, more patients with diabetic retinopathy were given treatment with insulin than patients without diabetic retinopathy. Furthermore, in the patients with the insulin treatment lower insulin secretion was associated with marked plasma glucose excursions and also with elevated GA (Koga *et al.*, 2010). Thus, lower insulin secretion may be associated with the development of diabetic retinopathy, although plasma insulin levels were not determined in the present study.

5.7 Metabolic syndrome in Type 2 Diabetes

The metabolic syndrome is a common metabolic disorder that results from the increasing prevalence of central obesity(Eckel *et al.*, 2005).The prevalence of MetS in this study was 4.7 % which is very low compared to higher prevalence in studies by Titty *et al.* (2008) and Nsiah *et al.* (2015). This low prevalence may be attributed to low numbers of diabetics with central obesity in this study. The syndrome is increasingly recognized as a risk factor for diabetes mellitus and cardiovascular disease (Isomaa *et al.*, 2001).

Poor glycaemic control and metabolic syndrome are all risk factors for CVD(Grundy *et al.*, 2004). The proportion of the diabetic patients with MetS with poor glycaemia was 85.7% using both criteria. Thus, about 86% of the patients with MetS may be associated with CVD by HbA1c and GA values. Thus the metabolic syndrome was associated with

worsening glycaemic control. This confirms earlier reports by Thorn *et al.* (2005)that metabolic syndrome is correlated with poor glycaemic control.

Although the results of this study may suggest that metabolic syndrome and renal dysfunction may be related due to the increased renal function parameters, it is difficult to draw any definitive conclusion concerning a cause-and-effect relationship. This is because many patients with the metabolic syndrome have diabetes and are obese, which are widely known risk factors for the development and progression of CKD (Levey *et al.*, 2005). Study by Chen *et al.* (2004)revealed that hypertension and fasting plasma glucose levels of >110 mg/dl were the individual traits of the syndrome and that they are associated with the greatest risk for microalbuminuria and a low GFR.Chen *et al.* (2004)again found that reduced HDL cholesterol or high TG levels were independently associated with a significantly increased risk for CKD. However, some data also suggest that other aspects of the metabolic syndrome may play an independent role in promoting renal damage(Muntner *et al.*, 2000).

5.8 Relationship between Glycemic indices (HbA1c and GA), hemoglobin and serum albumin

Glycated albumin correlated significantly with Glycated hemoglobin indicating a direct relationship among the patients with diabetes ($r=0.902$, $p<0.0001$) which is consistent with the reports by (Inaba *et al.*, 2007; Pu *et al.*, 2007).In some studies glycated albumin is suggested as an alternative marker for glycemic control in many diabetes complications, including nephropathy (Koga *et al.*, 2011), retinopathy (Okumura *et al.*, 2007) and also in the case of hemodialysis patients or gestational diabetes (Nagayama *et al.*, 2009; Hashimoto *et al.*, 2010). This observations further indicate that the determination of the serum Glycated albumin level may be a valuable adjunct to HbA1c

measurement for evaluating short-term glycemic control in diabetic patients (Pu *et al.*, 2007).

Some studies have reported that in some case, HbA1C values should be considered cautiously. As a matter of fact, glycated hemoglobin levels have invalid correlation to blood glucose levels in patients with hemolytic anemia, or those having hemodialysis or iron deficiency (Takahashi *et al.*, 2007; Hashimoto *et al.*, 2010). In the present study however, highly significant association was observed between Glycated albumin and serum albumin but different for Glycated hemoglobin and hemoglobin.

Thus in numerous case such as hemolytic or renal anemia and liver cirrhosis, HbA1c gives incorrect values and is not suitable marker as a control (Jeffcoate, 2004). Glycated albumin, because of its shorter half-life (21 days) compared with hemoglobin, could be used as a shorter-term glycemic control for diabetes. The glycated albumin level could not to be easily altered by abnormal hemoglobin metabolism (Kosecki *et al.*, 2005). This advantage of glycated albumin is based on two facts. First, the amount of in vivo non-enzymatic glycation of albumin is approximately 9 times more than HbA1C. Secondly, albumin glycation reaction occurs ten times more quickly than hemoglobin glycation so, the glycation phenomenon in plasmatic protein occurs more easily than hemoglobin, which all make the glycated albumin a good additional marker for evaluating glycemic control in type 1 and 2 diabetes (Adler *et al.*, 2000; Yoshiuchi *et al.*, 2008).

5.9 Risk factors associated with poor glycemic control using both criteria in T2DM

In the light of the present study, gender and obesity were shown to increase the odds of poor glycemic control assessed by both criteria. Inflammation is known to decrease the rate of albumin synthesis and increase its catabolic rate. Thus, chronic inflammation

process may provide a mechanism for increased turnover of serum albumin in obese subjects which may further influence GA levels (Schultze & Heremans, 1966; Don & Kaysen, 2004). This could however explain the positive correlation between GA and BMI in this study. On the contrary, Koga *et al.* (2006) found a significant negative correlation of GA levels and BMI and no correlation of BMI with HbA1c.

5.10 Predictive value of HbA1c and GA in the monitoring of t2dm

The findings in this study indicated that Glycated albumin (GA) as compared to HbA1c is a better marker of glycaemic control in monitoring T2DM. Several studies (Guthrow *et al.*, 1979; Koga *et al.*, 2010) have shown that GA is a more reliable DM monitor and a better marker of glycaemic control than is HbA1c in patients undergoing hemodialysis and in patients with fluctuating and poorly controlled type 2 DM. Measurements of glycated haemoglobin (HbA1c) and glycated albumin (GA) have been used clinically to monitor glycaemic control in patients with diabetes. HbA1c represents an integrated measurement of blood glucose during the preceding 2 months while serum GA, a shorterterm marker, reflects glycaemic control over approximately the preceding 2 weeks (Guthrow *et al.*, 1979; Shima *et al.*, 1988).

GA is not influenced by a number of physiologic and pathologic conditions that affect HbA1c levels, such as anemia and genetic haemoglobin abnormalities (Bry *et al.*, 2001).Unfortunately, there may also be interferences with the GA assay. While HbA1c measurement is affected by reduced erythrocyte survival or an increase in young erythrocytes (e.g., during treatment with erythropoietin stimulating agents), GA can be influenced by factors that affect albumin turnover (Koga *et al.*, 2007; Miyashita *et al.*, 2007). Since the half-life of serum albumin is around 2 weeks, shorter than that of erythrocytes, GA reflects shorter terms of glycaemic control than HbA1c (Tahara *et al.*,

1993). Reflecting such characteristics, it has been recently shown that changes in GA can predict change in HbA1c after diabetes treatment (Okada *et al.*, 2011; Won *et al.*, 2012). In addition, there have been accumulating evidences that HbA1c mainly reflects mean plasma glucose levels while GA also reflects plasma glucose excursions and/or postprandial glucose levels better than HbA1c (Cohen, 1988; Ogawa *et al.*, 2012).

CHAPTER 6

CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

Glycated albumin reflects glucose excursions more strongly than HbA1c, hence GA might be a more sensitive index for some diabetic complications than HbA1c. The available evidence points to glycated albumin levels as a useful marker for diabetes management, although much still needs to be learned about the mechanism of albumin glycation and about how this marker compares with the much better established HbA1c. However, the significant association between Glycated albumin and HbA1c suggests that serum Glycated albumin level may be a valuable adjunct to HbA1c measurement for evaluating short-term glycemic control in diabetic patients.

The results indicate that female diabetic patients turn to develop dyslipidemia and obesity than their male counterparts resulting in increased poor glycaemic control on the part of the females. TC, LDL was not significantly associated with GA and HbA1c levels, however, increase in TG levels may partly result in increased level of GA and HbA1c. This could however, suggest that diabetic patients with dyslipidemia may have difficulty controlling their blood glucose.

The finding of this study also provides evidence of poor renal function in the patients with diabetes and is further complicated by SCD. The disease complications evaluated

revealed 27.3% and 20.0% diabetic nephropathy and retinopathy respectively and were attributed 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. Hence the need for critical monitoring of patients with diabetes in other to curb the increase in the complications.

Factors associated with poor glycaemic control in diabetic patients included gender, obesity, disease complications and the state of their renal function. These factors however were determined to increase the odds of having poor glycaemic control assessed by GA and HbA1c.

6.2 RECOMMENDATION

There is therefore the need for critical monitoring of patients with diabetes in other to curb the increase in the complications.

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