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ASSOCIATION BETWEEN GLYCAEMIC CONTROL AND LIPID PROFILE AMONG TYPE 2 DIABETES PATIENTS: GLYCATED HAEMOGLOBIN AS A DUAL MARKER

BY
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THE ASSOCIATION BETWEEN GLYCAEMIC CONTROL AND LIPID PROFILE AMONG TYPE 2 DIABETES PATIENTS: GLYCATED HAEMOGLOBIN AS A DUAL MARKER

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DECLARATION

With the exception of the quoted statements and acknowledged sources, I hereby declare that this is an original piece of research work carried out in Koforidua Regional Hospital. This work has not been submitted for any other degree.

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DEDICATION

This thesis is dedicated to my entire family especially my little boy (Gilead) and my wife.

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ABSTRACT

Apart from classical risk factors like dyslipidemia, elevated Glycated Haemoglobin has now been regarded as an independent risk factor for cardiovascular disease in people with or without diabetes. Glycated hemoglobin (HbA1c) is a routinely used marker for long-term glycemic control. In accordance with its function as an indicator for the mean blood glucose level, HbA1c predicts the risk for the development of diabetic complications in diabetes patients. The study was aimed at investigating the relationship between glycemic control and serum lipid profile and to evaluate the role of Glycated Haemoglobin as an independent risk factor for cardiovascular diseases in patients with type-2 diabetes. In all 200 diabetic patients who were under treatment were randomly sampled for the study. Socio- demographic data were collected using predesigned questionnaires. Glycated haemoglobin levels, lipid profile, fasting blood sugar, haemoglobin and red blood cell count were estimated using standard procedures. Anthropometric variables such body mass index (BMI), visceral adiposity index (VAI) and body adiposity index (BAI) were measured. Systolic and diastolic blood pressures were also taken. Dyslipidemia was defined as per the National Cholesterol Education Programme (NCEP) Adult Treatment Panel (ATP) III guidelines. Diabetes was defined as per American diabetes association (ADA) criteria. The Statistical analysis was done by SPSS statistical package version 20.0. The patients were divided into two groups (HBA1C ≤7.0% and >7.0%). Duration of type 2 diabetes had a positive correlation with glycated haemoglobin. Females had significantly higher BMI (p= 0.0002), BAI (p= 0.0008), hip circumference (p= 0.0002), and waist circumference (p= 0.0008) measurements than their male counterparts. Participants with higher HBA1C (> 7.0%) levels also had significantly higher measurements in BMI and BAI as compared with those of desirable HBA1C (≤7.0%) levels. The levels of HbA1c and FBG did not differ significantly between male and female. Although there were no significant difference in TC (p=0.2287), LDL-C (p=0.3821) and HDL-C (p=0.5248) levels between male and female, the levels of TG were significantly higher (p=0.0059) in female as compared to male type 2 diabetic patients. This study reveals high prevalence of hypercholesterolemia, hypertriglyceridemia, high LDL-C and low HDL-C levels which are well known risk factors for cardiovascular diseases. Significant correlations were observed between HbA1c and TC, LDL-C and TG. Significant correlation between HbA1c and various circulating lipid parameters and significant difference of lipid parameters in two groups (≤7.0% and >7.0%) of glycated hemoglobin indicates that HbA1c can be used as a potential biomarker for predicting

dyslipidemia in type 2 diabetic patients in addition to glycemic control. Hence early diagnosis can be accomplished through relatively inexpensive blood testing and may be utilized for screening high-risk diabetic patients for timely intervention with lipid lowering drugs.

ABBREVIATIONS

ADA	American Diabetes Association
ATP III	Adult Treatment Panel III
AMORIS	Apolipoprotein-Related Mortality Risk
BAI	Body Adiposity Index
BMI	Body Mass Index
CHD	Coronary Heart Disease
CETP	Cholesteryl Ester Transfer Protein
CD	Cluster of Differenciation
CVD	Cardiovascular Disease
CRP	C- Reactive Protein
DM	Diabetic Mellitus
DCCT	Diabetes Control and Complication Trial
EDIC	Epidemiology of Diabetes Intervention and Complication
FFA	Free Fatty Acid
GAD	Glutamic-acid-decarboxylase
HBAIC	Glycated Haemoglobin
HSL	Hormone-sensitive Lipase
HDL-C	High Density Lipoprotein Cholesterol
HC	Hip Circumference
IDF	International Diabetic Federation
IL-6	Interleukin 6
IGT	Impaired Glucose Tolerance
IDDM	Insulin Dependent Diabetes Mellitus

LDL-C	Low Density Lipoprotein Cholesterol
LPL	Lipoprotein Lipase
NIDDM	Non-Insulin Dependent Diabetes Mellitus
NHDL-C	Non High density lipoprotein cholesterol
NGSP	National Glycohaemoglobin Standardisation Programme
NCEP	National Cholesterol Education Program
OGTT	Oral Glucose Tolerance Test
TG	Triglycerides
TC	Total Cholesterol
T2DM	Type 2 Diabetes Mellitus
UKPDS	United Kingdom Prospective Diabetes Study
VAI	Visceral Adiposity Index
VLDL-C	Very Low Density Lipoprotein Cholesterol
VAT	Visceral Adipose Tissue
W.H.O	World Health Organization
WTHR	Waist to Hip Ratio
WC	Waist Circumference

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Chapter 1

INTRODUCTION

1.1 BACKGROUND

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Type 2 diabetes, the most prevalent form of the disease, is often asymptomatic in its early stages and can remain undiagnosed for many years (American Diabetes Association, 2003).

The International Diabetes Federation (IDF) in 2014 reported that 450,000 people (3.35% prevalence) have been diagnosed as having diabetes with a further 330,000 undiagnosed persons in Ghana alone (International Diabetes Federation, 2014). The prevalence of DM has increased dramatically around the globe, from an estimated 30 million cases in 1985 to 177 million in 2000. It is estimated that, more than 360 million individuals may develop diabetes by the year 2030 (Fauci, 2008).

Diabetic patients have a greater likelihood of having dyslipidemia, hypertension, and obesity. Because early detection and prompt treatment may reduce the burden of diabetes and its complications, screening for diabetes may be appropriate under certain circumstances (American Diabetes Association, 2003).

Epidemiological studies have demonstrated that type 2 diabetes mellitus (DM) is a well-known risk factor for the development of cardiovascular disease, cerebrovascular disease and peripheral vascular diseases. Dyslipidemia is a risk factor for coronary

artery disease, a leading cause of mortality in patients with diabetes mellitus. Dyslipidemia remains largely undiagnosed and under treated in high risk populations, such as patient with type- 2 diabetes (Masram *et al*., 2012).

Glycated hemoglobin (HbA1c) is a routinely used marker for long-term glycemic control. In accordance with its function as an indicator for the mean blood glucose level, HbA1c predicts the risk for the development of diabetic complications in diabetes patients. Apart from classical risk factors like dyslipidemia, elevated HbA1c has now been regarded as an independent risk factor for cardiovascular disease in subjects with or without diabetes (Khaw *et al*., 2004).

Estimated risk of cardiovascular disease has shown to be increased by 18% for each 1% increase in absolute HbA1c value in diabetic population. Positive relationship between HbA1c and cardiovascular disease has been demonstrated in non-diabetic cases even within normal range of HbA1c (Ram *et al*., 2011).

1.2 STATEMENT OF PROBLEM

Ghana has a high diabetes prevalence rate of 3.35% with more than 8,500 diabetes related death each year (International Diabetes Federation, 2014). These diabetic patients with accompanied (but often unnoticed) dyslipidemia are soft targets of cardiovascular deaths. Patients with type 2 diabetes often exhibit an atherogenic lipid profile, which greatly increases their risk of cardiovascular disease compared with people without diabetes. This form of dyslipidemia remains largely undiagnosed or diagnosed late and under treated in high risk populations, such as patient with type-2 diabetes.

Proper monitoring and treatment of glycemia and its complications in diabetic patients has been the key in reducing diabetes related deaths. HbA1c has been the traditional marker for assessing long term glycemic control and can also predict the onset of dyslipidemia which increases the risk of cardiovascular disease (Ram *et al*., 2011).

Nevertheless it has received some criticisms over its inability to measure accurately glycation and predict dyslipidemia among diabetic patients in certain haematological and biochemical conditions (Speeckaert *et al*, 2014). Also there is paucity of data on the use of glycated haemoglobin and its effectiveness in glycemic monitoring and its association with lipid profile of diabetic patients.

1.3 GENERAL AIM OF STUDY

The aim of the study is to investigate the relationship between glycemic control and serum lipid profile and to evaluate the role of HbA1c as an independent risk factor for cardiovascular diseases in type-2 diabetic patients.

1.4 SPECIFIC OBJECTIVES OF STUDY

The specific objectives of the study are:

1. To investigate the association between glycated haemoglobin and some anthropometric measurement of obesity.
2. To investigate the association between Glycated Haemoglobin, Fasting blood sugar and Lipid profile

3. To investigate whether Glycated haemoglobin is a risk factor for cardiovascular disease.

1.5 JUSTIFICATION OF RESEARCH

Apart from classical risk factors like dyslipidemia, elevated HbA1c has now been identified as an independent risk factor for cardiovascular disease in subjects with or without diabetes. Estimated risk of cardiovascular disease has shown to be increased by 18% for each 1% increase in absolute HbA1c value in diabetic population (Ram *et al*., 2011). Although new tests of glycation are now available, they are not without limitations of their own (Speeckaert *et al*, 2014), very expensive and impractical for everyday use in developing countries like Ghana. However the routinely used biomarker, HbA1c which is relatively cheap and affordable (in a developing country like Ghana) has come under some critiques for not properly assessing the level of glycation and also dyslipidemia in certain haematological and biochemical conditions. This study is an attempt to reassess the association of glycemic control and lipid profile using HbA1c whiles taking into consideration some of the factors affecting its use. This study will also afford health care providers attending to diabetic patients, the needed information as to when to use HbA1c in clinical monitoring of glycation and dyslipidemia.

Chapter 2

LITERATURE REVIEW

2.1 CLASSIFICATION OF DIABETES

Classification of diabetes includes both aetiological types and different clinical stages of hyperglycaemia as suggested by Kuzuya and Matsuda (2007). Four main aetiological categories of diabetes have been identified as diabetes type 1, type 2, other specific types, and gestational diabetes.

2.1.1 Type 1 Diabetes

Type 1 diabetes characterized by deficiency of insulin due to destructive lesions of pancreatic b-cells; usually progresses to the stage of absolute insulin deficiency. Typically, it occurs in young people with acute-onset with typical symptoms of diabetes together with weight loss and propensity to ketosis, but type 1 diabetes may occur at any age, sometimes with slow progression (Laakso and Pyorala, 1985). People who have antibodies to pancreatic b-cells such as glutamic-acid-decarboxylase (GAD), are likely to develop either typical acute-onset or slow-progressive insulin dependent diabetes (Gottsater *et al*., 1993; Tuomilehto *et al*., 1994). Today antibodies to pancreatic b-cells are considered as a marker of type 1 diabetes, although such antibodies are not detectable in all patients.

2.1.2 Type 2 Diabetes

Type 2 diabetes is caused by a combination of decreased insulin secretion and decreased insulin sensitivity. Typically, the early stage of type 2 diabetes is characterized by insulin resistance and decreased ability for insulin secretion causing excessive post-prandial hyperglycaemia. This is followed by a gradually deteriorating

first-phase insulin response to increased blood glucose concentrations (Bruce *et al*., 1988).

Type 2 diabetes, comprising over 90% of adults with diabetes, typically develops after middle age. The patients are often obese or have been obese in the past and have typically been physically inactive. Ketoacidosis is uncommon, but may occur in the presence of severe infection or severe stress.

2.1.3 Gestational Diabetes

Gestational diabetes constitutes any glucose perturbation that develops during pregnancy and disappears after delivery. Long-term follow-up studies, recently reviewed by Kim et al., reveal that most, but not all, women with gestational diabetes do progress to diabetes after pregnancy**.** In some cases, type 1 diabetes may be detected during pregnancy (Kim *et al*., 2002). However women who had diagnosed diabetes before pregnancy cannot be said to have gestational diabetes. The definition applies regardless of the type of treatment needed during the course of the pregnancy and whether the patient remains diabetic after delivery.

2.1.4 Other Types

Other types include: (i) diabetes related to specific single genetic mutations that may lead to rare forms of diabetes, as for instance Maturity Onset Diabetes of the Young (MODY); (ii) diabetes secondary to other pathological conditions or diseases (as a result of pancreatitis, trauma, or surgery of pancreas); (iii) drug or chemically induced diabetes.

The clinical classification also comprises different stages of hyperglycaemia, reflecting the natural history of absolute or relative insulin deficiency progressing from normoglycaemia to diabetes. It is not uncommon that a non-diabetic individual may move from one category to another in either direction. Usually, a progression towards a more severe glucose abnormality takes place with increasing age. This is reflected by the increase in the 2-hPG level with age (The DECODE Study Group, 2003).

The currently valid clinical classification criteria have been issued by WHO and ADA (Genuth *et al*., 2003). The WHO recommendations for glucometabolic classification are based on measuring both fasting and 2 hour post prandial glucose (2-hPG) concentrations and recommend that a standardized 75 g OGTT should be performed in the absence of overt hyperglycaemia (World Health Organization, 1999). The thresholds for diabetes on fasting and 2-hPG values were primarily determined by the values where the prevalence of diabetic retinopathy, which is a specific complication of hyperglycaemia, starts to increase. Even though macrovascular diseases such as CHD and stroke are major causes of death in type 2 diabetic patients and people with IGT, macrovascular disease has not been considered in the classification. This sounds illogical and may give an impression that macrovascular diseases are less important than microvascular consequences of diabetes.

Classification according to the ADA criteria strongly encourages the single use of fasting glycaemia only without an OGTT (Report of the Expert Committee, 1997).

Table 2.1: Criteria used for Glucometabolic according to WHO (1999) and ADA (1997) and (2003).

Glucometabolic category	Source	Classification criteria mmol/L (mg/dL)
Normal glucose regulation (NGR)	WHO	FPG < 6.1 (110) + 2-h PG < 7.8 (140)
	ADA (1997)	FPG < 6.1 (110)
	ADA (2003)	FPG < 5.6 (100)
Impaired fasting glucose	WHO	FPG ≥ 6.1 (110) and < 7.0 (126) + 2-h PG < 7.8 (140)
	ADA (1997)	FPG ≥ 6.1 (110) and < 7.0 (126)
	ADA (2003)	FPG ≥ 5.6 (100) and < 7.0 (126)
Impaired glucose tolerance (IGT)	WHO	FPG < 7.0 (126) + 2-h PG ≥ 7.8 and < 11.1 (200)
Impaired glucose homeostasis (IGH)	WHO	IFG or IGT
Diabetes mellitus (DM)	WHO	FPG ≥ 7.0 (126) or 2-h PG ≥ 11.1 (200)
	ADA (1997)	FPG ≥ 7.0 (126)
	ADA (2003)	FPG ≥ 7.0 (126)

Values are expressed as venous plasma glucose.
FPG = fasting plasma glucose; 2-h PG = two-hour post-load plasma glucose (1 mmol/L = 18 mg/dL).

Source: (Guidelines on diabetes, pre-diabetes, and cardiovascular diseases, 2007)

2.3 COMPLICATIONS OF DIABETES

2.3.1 Acute Complications

The usual clinical symptoms of DM include polyuria, polydipsia, weight loss, fatigue, weakness, blurred vision, frequent superficial infections, and poor wound healing. However, patients can occasionally present with acute complications, such as hypoglycaemia, diabetic ketoacidosis, hyperosmolar non-ketotic coma (Boon, 2006; Fauci, 2008).

2.3.2 Chronic Complications

The major chronic complications of DM are usually microvascular, neuropathic and macrovascular in nature. The microvascular and neuropathic complications present as retinopathy, nephropathy, peripheral neuropathy, autonomic neuropathy and foot disease. The macrovascular complications present as myocardial infarction/ ischaemia, transient ischaemic attack, stroke and claudication (Boon, 2006). Fauci (2008) have also indicated that other chronic complications of diabetes may be nonvascular, e.g. gastroparesis, infections and skin changes.

2.3.2.1 Macrovascular Complications

Boyle (2007) stated that in patients with type 2 diabetes, there is an increased risk of macrovascular disease. Factors that may play a linkage role in the development of macrovascular disease in type 2 diabetes include low concentration of the adipocyte specific protein (adiponectin) , increased production of the vascular cell adhesion molecule-1 and the subsequent adhesion of T-lymphocytes to the endothelial walls of the coronary arteries, higher procoagulation with increased expression of plasminogen activator inhibitor-1 (PAI)-1, and an increased production of matrix metalloproteinases (MMPs) by macrophages which ultimately leads to an instability of atherosclerotic plaques.

Almdal *et al* (2004) also indicated that Type 2 diabetes typically occurs in the setting of the metabolic syndrome, which also includes abdominal obesity, hypertension, hyperlipidaemia, and increased coagulability. These other factors can also act to promote cardiovascular disease. In this setting of multiple risk factors, type 2 diabetes itself acts as an independent risk factor for the development of ischemic disease, stroke, and death.

2.3.2.2 Retinopathy

Diabetic retinopathy which occurs in all forms of diabetes is the commonest cause of blindness in adults in most developed countries. The development of retinopathy, as with all diabetic complications, depends on the duration of the disease.

The natural history of diabetic retinopathy according to Nathan (1993) has been best defined in IDDM, where it is possible to predict the date of onset of the disease. Course of progression of retinopathy in NIDDM is more difficult to ascertain, since the diabetes may be progressing silently for many years before it is diagnosed. As a result, patients with NIDDM may present with retinopathy and even, rarely, advanced retinopathy at the time of diagnosis. However, the development of retinopathy is still dependent on how long the patient has had NIDDM, as it is in IDDM.

The clinical features of diabetic retinopathy are; 'microaneurysms, retinal haemorrhages, exudates, cotton wool spots, neovascularisation, fibrosis, pre-retinal and vitreous haemorrhages. These features occur in various combinations in different patients and are used to classify the severity of the disease' (Boon 2006).

2.3.2.3 Neuropathy

This is a relatively early and common complication affecting approximately half of all patients diagnosed with both types 1 and 2 diabetes. Fauci (2008) asserts that the condition may manifest as polyneuropathy, mononeuropathy, and/or autonomic neuropathy. As with other complications of DM, the development of neuropathy largely depends on duration of diabetes and how well or otherwise glucose levels are controlled. Also increased body mass index and smoking are considered risk factors for developing the complication.

2.4 DIABETIC DYSLIPIDEMIA

A characteristic pattern, termed diabetic dyslipidemia, consists of specifically mild to marked elevation of triglyceride-rich lipoproteins (VLDLs) and VLDL remnants concentrations and low levels of HDL-C. Raised serum triglycerides and low HDL-C often precede the onset of T2DM for many years. In addition, LDL particles are converted to smaller, perhaps more atherogenic, lipoproteins termed 'small-dense LDLs' (Fruchart *et al*., 2008, Betteridge and Morrell, 1998).

Different mechanisms are responsible for the development of dyslipidemia in individuals with diabetes. Defects in insulin action and hyperglycemia could lead to dyslipidemia in patients with diabetes. In the case of T2DM, the obesity/insulinresistant state that is at the basis of the development of this disease can in itself lead to lipid abnormalities independently of hyperglycemia. In poorly controlled T1DM hypertriglyceridemia and reduced HDL-C commonly occur, but in most cases insulin replacement in these patients correct these abnormalities. In T2DM, this phenotype is not usually fully corrected with glycemic control, suggesting that insulin resistance and not hyperglycemia *per se* are associated with this lipid abnormality (Durrington and Sniderman, 2002).

Insulin-controlled apoprotein production in the liver, regulation of lipoprotein lipase (LPL), actions of cholesteryl ester transfer protein (CETP) and peripheral actions of insulin on adipose tissue and muscles are considered to be important mechanisms responsible for diabetic dyslipidemia (Durrington and Sniderman, 2002; Stralfors and Honnor, 1989).

2.4.1 Epidemiology Of Diabetic Dyslipidemia

In the Framingham Heart Study (Kannel, 1985), 13% of men and 24% of women with diabetes mellitus had increased total plasma cholesterol levels, compared with 14% of men and 21% of women without diabetes mellitus. The prevalence of high LDL cholesterol levels in men and women with diabetes mellitus (9% and 15%, respectively) did not differ significantly from the rates in non diabetic men and women (11% and 16%, respectively). By contrast, the prevalence of high plasma triglyceride levels in individuals with diabetes mellitus (19% in men and 17% in women) was significantly higher than in those without diabetes mellitus (9% of men and 8% of women).

High levels of total cholesterol, LDL cholesterol and triglyceride were defined as values above the corresponding 90th percentile for the US population. The prevalence of low HDL cholesterol level (defined as a value below the 10th percentile for the US population) in those with diabetes mellitus was almost twice as high as the prevalence in nondiabetic individuals (21% versus 12% in men and 25% versus 10% in women, respectively) (Kannel, 1985).

Thus, both men and women with diabetes had an increased prevalence of hyper trigyceridemia and low HDL cholesterol levels, but their total cholesterol and LDL cholesterol levels did not differ from those in non-diabetic counterparts (Kannel, 1985). A similar pattern of altered plasma lipid profiles was observed in the UK Prospective Diabetes Study (UKPDS).

2.4.2 Pathophysiology of Diabetic Dyslipidemia

The precise pathogenesis of diabetic dyslipidemia is not known; nevertheless, a large body of evidence suggests that insulin resistance has a central role in the development of this condition (Taskinen, 2003; Krauss and Siri, 2004; Del Pilar Solano and Goldberg, 2005; Chahil and Ginsberg, 2006).

The main cause of the three cardinal features of diabetic dyslipidemia is the increased free fatty-acid release from insulin-resistant fat cells (Taskinen, 2003; Krauss and Siri, 2004; Del Pilar Solano and Goldberg, 2005; Chahil and Ginsberg, 2006).

Although DM and dyslipidemia are two separate risk factors for atherosclerosis, the results of many studies on patients with T2DM show that diabetes and dyslipidemia have a whole range of pathophysiological overlaps and that these interactions accelerate the process of atherogenesis (Betteridge and Morrell, 1998; Isomaa *et al*., 2001) as a result, they should not be observed separately for many reasons. Presenting at the same time they do not only exponentially increase cardiovascular risk, but form a vicious circle in which dyslipidemia worsens diabetes and diabetes worsens dyslipidemia.

The impact of diabetes and dyslipidemia on the development of atherosclerotic plaque was studied by Drexel *et al*. (2005) Their aim was to evaluate the atherogenic effect of individual lipid fractions in individuals with normal blood glucose, in those with impaired glucose tolerance, and in patients with T2DM. Serum lipid concentration, the presence of angiographically proven CHD at the beginning of the study, and the incidence of vascular events were followed in 750 individuals undergoing coronarography during a 2.3-year period. Blood glucose concentration correlated

negatively with low HDL levels and positively with triglyceride levels. In patients with T2DM and CHD, such a finding is associated with the incidence of CHD and is a major predictor of coronary events.

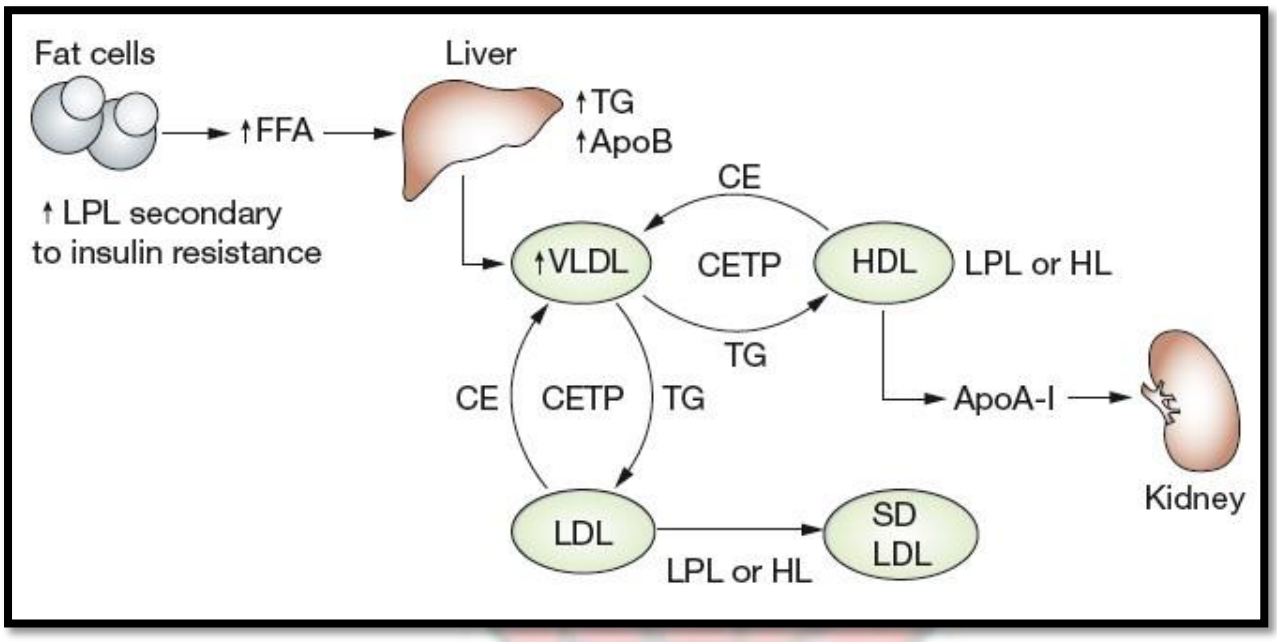
Some studies have observed a significantly increased expression of CD36 scavenger receptors, previously known as receptors for advanced glycation end products, in patients with glucose metabolism disorder (Fuchs, 2008). They provided evidence that the degree of accumulation of oxidized LDL-C depends on blood glucose levels. Macrophages that were differentiated from human peripheral blood monocytes in the presence of high glucose concentrations showed increased expression of cell-surface CD36 secondary to an increase in translational efficiency of CD36 mRNA. They have also observed significant expression of CD36 scavenger receptors in vascular lesions of patients with increased blood glucose values when compared with normoglycemic individuals, emphasizing their possible role in foam cell formation (Fuchs, 2008).

Insulin is a pleiotropic molecule that has effects on amino acid uptake, protein synthesis, proteolysis, adipose tissue triglyceride lipolysis, LPL activity, VLDL triglyceride secretion, muscle and adipose tissue glucose uptake, muscle and liver glycogen synthesis, and endogenous glucose production. T2DM is a heterogeneous disease of polygenic origin, which is characterized by impaired insulin secretion and peripheral insulin resistance. β-cell dysfunction is always present. The nature of the β-cell defect has not been fully clarified thus far. In physiological circumstances insulin reduces VLDL particles and triglycerides, as well as ApoB production and secretion (Lewis and Steiner, 1996). Another intrahepatic effect of insulin is to enhance ApoB degradation (Lewis and Steiner, 1996; Cornier *et al.*, 2008).

When insulin resistance is present, or when insulin secretion is diminished in the later stages of the disease, FFAs are released in large quantities followed by an increased production of glucose and triglycerides, and secretion of VLDL occurs. In addition, FFAs also reduce insulin sensitivity in muscles by inhibiting insulin-mediated glucose uptake. On the other hand, increased blood glucose concentration, and to some extent circulating FFA, increase insulin secretion, leading to even more increased hyperinsulinemia. It is obvious that insulin resistance causes blood glucose concentrations and FFA levels to rise, therefore worsening the insulin resistance and, in addition, hyperglycemia with released FFA further increasing insulin secretion, forming a vicious circle (McGarry, 2002; Pan *et al.*, 1997).

It has been hypothesized that triglyceride accumulation in skeletal muscles plays a direct role in the etiology of insulin resistance (Fruchart *et al.*, 2008). The results of some studies have shown that the degree of insulin resistance is positively correlated with intramuscular triglycerides content (Poitout and Robertson, 2002). According to Poitout and Robertson, chronic hyperglycemia and dyslipidemia in T2DM can both produce harmful effects on β-cell structure and function. Although inter-relationships between glucotoxicity and lipotoxicity have not yet been elucidated, it is presumed that glucotoxicity leading to β-cell apoptosis occurs independently of dyslipidemia, whereas lipotoxicity additionally damaging β-cells occurs only in the presence of hyperglycemia. In the case of normoglycemia, elevated FFAs should be readily oxidized in the mitochondrion and should not harm the β-cell (Colhoun *et al.*, 2004).

Figure 2.1 the role of insulin resistance in diabetic dyslipidemia.



Source: (Mooradian, 2008)

2.4.3 Triglyceride-rich Lipoproteins and Triglycerides in T2DM

In patients with uncontrolled T2DM and hyperinsulinemia, triglycerides are elevated for several reasons. The release of stored fatty acids (FAs) from adipocytes requires the conversion of stored triglycerides into FAs and monoglycerides that can be transferred across the plasma membrane of the cell. The primary enzyme that is responsible for this is hormone-sensitive lipase (HSL), which is normally inhibited by insulin. In insulin resistance, HSL becomes active, resulting in increased free fatty acids (FFAs) being delivered from adipose tissue to the liver, giving rise to hepatic overproduction of large VLDL particles and hypertriglyceridemia (Durrington and Sniderman, 2002).

In addition to LPL an enzyme that plays a key role in breaking down triglycerides present in chylomicrons and VLDL particles, is less effective in the insulin-resistant

state and triglyceride clearance from the circulation is diminished (Lewis and Steiner, 1996).

Patients with poorly controlled T1DM and T2DM often have increased VLDLcholesterol concentration. Diminished insulin action influences apolipoproteinB (Apo B) synthesis, while increased activity of HSL leads to enhanced influx of FFAs through the portal vein system.

In diabetes, greater amounts of FFAs returning to the liver are reassembled into triglycerides and secreted in VLDL. A greater content of triglyceride leads to the production of larger particles. Both the composition and the size of VLDL determine its metabolic rate, so not all VLDLs are equally likely to be converted into LDLs. A great proportion of large, lighter VLDLs return to the liver without complete conversion to LDL (Dixon *et al*., 1991).

Another mechanism implicated in increased VLDL production in T2DM is increased liver production of Apo B, the major protein component of VLDLs and LDLs. Most of the newly synthesized protein is degraded either during or immediately after translation, while this degradation can be prevented with the addition of lipids to the protein through the action of microsomal triglyceride transfer protein (Stralfors and Honor, 1989). The results of different studies suggest that FAs modulate liver Apo B secretion. Thus, lipid concentration in the liver regulates ApoB production. Decreased insulin secretion in diabetes characterized by increased lipolysis in adipocytes, increased FA release from fat cells and increased return to the liver, support this hypothesis (Stralfors and Honor, 1989).

2.4.4 LDLs in Diabetes

Mixed or combined hyperlipidemia is common in patients with diabetes. In addition to cholesterol, VLDL particles of diabetic patients also contain a larger quantity of triglycerides. Small dense LDLs are mainly generated by the following mechanism: composition and the size of the VLDL determines its metabolic fate. Due to the elevation of triglyceride concentration, both VLDLs and chylomicron particles remain in circulation for longer periods. This allows an increased transfer of cholesterol esters. CETP facilitates the transport of cholesteryl esters and triglycerides between lipoproteins. It collects triglycerides from VLDL or LDL particles and exchanges them for cholesteryl esters from HDLs, and vice versa. Increased activity of CETP under these conditions drives the production of small, dense atherogenic triglyceride-rich LDL particles (Stralfors and Honor, 1989). They are a substrate for the hepatic lipase (HL) enzyme, which usually has an elevated activity in patients with T2DM (Lee *et al*., 1990)

The end product of this process is the formation of small dense LDL particles. The characteristics of these small dense LDL particles is reduced binding affinity for the LDL receptor, resulting in prolonged plasma residence time, a greater ability to penetrate the arterial wall compared with larger LDL particles, and increased susceptibility to oxidation, leading to accelerated atherogenesis (Fruchart *et al*., 2008). The Strong Heart Study showed that there is a stepwise decrease in LDL size according to diabetic status from normal to impaired glucose tolerance, and then to diabetes (Gotto *et al*., 2000). This association is more striking in women than in men, which may be the reason why diabetic women lose their sex-specific cardioprotection

(Gotto *et al*., 2000; Taskinen, 2002**)** even at concentrations well below the National Cholesterol Education Program (NCEP) target (Graham *et al*., 2007).

Low density lipoprotein-cholesterol is a strong independent predictor of CHD in patients with DM, particularly when components of diabetic dyslipidemia are present. These results support recent recommendations for the aggressive control of LDL-C in diabetic individuals, with a target level of <80 mg/dl (2.0 mmol/l) (Walldius *et al*., 2001).

Since an increased number of small dense LDL particles is particularly dangerous, Apo B measurement in these patients should be advised. The Apolipoprotein-Related Mortality Risk (AMORIS) study was specifically designed to compare cholesterol and Apo B as markers of the risk of death of myocardial infarction, showing that Apo B was a superior marker for risk estimation to total or LDL-C in every direct comparison. Several studies indicate that Apo B/Apo A-I ratio is superior to the total cholesterol/HDL-C ratio. **(**Durrington and Sniderman, 2002**;** Lewis and Rader, 2005)

2.4.5 HDLs in Diabetes

Low levels of HDL-C are commonly found in association with hypertriglyceridemia, a pattern that is commonly observed in patients with T2DM. A low level of HDL-C under 40 mg/dl (<1 mmol/l) is an independent risk factor for CVD. In diabetic dyslipidemia, the concentration of HDL-C is reduced and its compositions as well as distribution are changed. The electrophoretic spectrum shows a shift towards smaller

HDL particles and HDL_2 is reduced (Reiner, 2009; Verges *et al*., 1992). Vergles *et al.* investigated the influence of hypertriglyceridemia and obesity in men and women with T2DM. When one or both of the factors were present, the HDL_2-C levels were significantly lower in the diabetic population when compared with controls. However, when obesity and hypertriglyceridemia were absent HDL_2-C levels in the diabetic population were not significantly different from controls (Rashid *et al*., 2002).

Changes in HDL in T2DM are mediated via two pathways: plasma triglyceride elevation, and a reduced ratio between LPLs and HL. Both lead to a modulation of HDL composition with an enhanced catabolic rate of HDL in the circulation. This process results in lower HDL-C. Moreover, the potent antiatherogenic activity of small dense, lipid-poor HDL_3 particles is attenuated by triglyceride enrichment mediated via CETP and subsequent hydrolysis of triglycerides by HL. ApoAI are unstable in these functionally defective small dense HDLs, making them prone to degradation and rapid elimination via the kidney. This leads to the low levels of HDL-C typically exhibited in T2DM (Fruchart *et al*., 2008).

High-density lipoproteins in the basal state contain enzymes that can destroy oxidized lipids, which mediate a chronic inflammatory response. Three main mechanisms responsible for combating oxidative stress in insulin-resistant individuals have been described:

- ApoA-I within HDL acts to attenuate lipid peroxidation;
- HDL-associated enzymes cleave lipid peroxides;
- Lipophilic antioxidants contained within HDL particles scavenge oxygen free radicals (Kuntush and Chapman, 2006; Benedict *et al*., 2009)

These mechanisms can be impaired in DM owing to the action of some acute-phase reactants. T2DM is associated with increased blood concentrations of markers of chronic low-grade inflammation or the acute-phase response markers (typically of the order of 1.5- to twofold). These include sialic acid, α-1 acid glycoprotein, serum amyloid A (SAA), C-reactive protein (CRP) and cortisol, and IL-6 (Betteridge and Morrell, 1998). The increased concentration of IL-6 can be connected with obesity and is common in patients with T2DM because it is also produced by adipocytes (Peter *et al*., 2008). This is thought to be a reason why obese individuals and individuals with T2DM often have higher endogeneous levels of CRP. Because of its stimulatory effect on the secretion of some acute-phase proteins, IL-6 is considered as the main cytokine mediator of acute-phase response. **(**Muačević-Katanec *et al*., 2007**)**

Two acute-phase reactants are known to interact with lipoproteins: CRP and SAA. CRP binds to lipoproteins containing ApoB, and SAA is found in the circulation almost exclusively associated with HDL. HDL taken up during the acute phase actually enhanced LDL-induced monocyte migration into the subendothelial space of the cocultures and was not effective in preventing lipid hydroperoxide formation. After incubation with purified SAA, ApoA-I content had decreased by 87%, while the activity of paraoxonase (PON1), an important antioxidant that prevents LDL peroxidation, (Van Lentel *et al*., 1995; Abbott *et al*., 1995) had decreased by 91% (Fruchart *et al*., 2008).

Reduced capacity of HDL particles to protect LDLs against oxidative modification in diabetes could also be explained by PON1 inactivation by some other mechanisms. According to the results of Abbott *et al.(1995)*, a larger proportion of the PON1 protein

could be inactive in diabetes, either because of the presence of an endogenous circulating inhibitor or because of increased glycation of PON1. The strong correlation between PON1 concentration and glycation of ApoA-I has been found in different studies (La Du and Novais, 1989).

The loss of this correlation in diabetic patients might indicate a disruption in the interaction between PON1 and the HDL particle. As described earlier, HDL from diabetic patients has a different structure and functionality compared with the HDL from healthy subjects. This could be the reason for defective binding of PON1 to such HDLs, resulting in increased LDL modification by lipid peroxides, which leads to accelerated atherosclerosis (Lee *et al*., 1990).

2.4.6 Treatment of Dyslipidemia in Diabetes

To achieve target values of lipids in patients with DM, aggressive treatment with all available means is often necessary. Using them simultaneously will be helpful in achieving improvement of the lipid status more quickly by different pharmacological mechanisms. Aggressive therapy of diabetic dyslipidemia will clearly reduce the risk of CVD in patients with DM. This has been shown in the Collaborative Atorvastatin Diabetes Study (CARDS), which has pointed to a need to consider patients with T2DM as candidates for statin treatment even at a lower LDL-C level goal (Knopp *et al*., 2006).

Although the Atorvastatin Study for Prevention of Coronary Heart Disease End Points in Non-Insulin-Dependent Diabetes Mellitus (ASPEN) could not demonstrate any significant difference in primary and individual end points for all patients, it seems that the degree of risk factors (e.g., blood pressure and smoking history) appears to

play a vital role in the extent to which statin therapy reduces CV risk in T2DM without CHD (American Diabetes Association, 2004).

Diet, weight reduction and regular physical activity have been considered to be a cornerstone of T2DM treatment. However, there is little evidence from clinical trials to prove the effect of different dietary interventions and physical activity on the incidence of cardiovascular events. Observational studies suggest that patients who report healthier diets and more intensive physical activity have fewer cardiovascular events (American Diabetes Association, 2004; Isomaa *et al.*, 2001).

There are different possible approaches to dyslipidemia treatment in patients with T2DM. Primary therapy should be directed towards lowering LDL-C levels. A statin should be chosen depending on the LDL-C reduction needed to achieve the target value (<80 mg/dl or <2.0 mmol/l) and upon the judgment of the physician. Although usually not very effective for raising HDL-C levels, statins might be effective at reducing moderately elevated triglycerides, thus reducing the need for combination therapy.

Achieving satisfactory glucoregulation can be very effective for reducing triglycerides levels. After achieving glucoregulation, the need for adding a fibrate, usually fenofibrate, for triglyceride lowering and/or niacin for HDL-C increase should be estimated. The disadvantages of niacin are side effects such as flushing, which seriously affects compliance, and also the fact that niacin therapy is accompanied by increases in glycemia. However, it seems that the latter is generally amenable to adjustment of glucose-lowering therapy. A problem might also be that new-onset DM

can occur after the initiation of niacin therapy in individuals with the metabolic syndrome, particularly if they have impaired glucose tolerance. Gemfibrozil can be prescribed to patients with T2DM with increased triglycerides, but should not be recommended to patients who have increased concentrations of both triglycerides and LDL-C as a combination therapy with a statin because of much higher risk of myopathy as an adverse effect (American Diabetes Association, 2004).

2.5 GLYCATED HAEMOGLOBIN (HbA1c)

HbA1c was introduced into clinical use in the 1980s and subsequently has become a cornerstone of clinical practice (Massi- Benedetti, 2006). HbA1c reflects average plasma glucose over the previous eight to 12 weeks (Nathan *et al*., 2007). It can be performed at any time of the day and does not require any special preparation such as fasting. These properties have made it the preferred test for assessing glycaemic control in people with diabetes. More recently, there has been substantial interest in using it as a diagnostic test for diabetes and as a screening test for persons at high risk of diabetes (International Expert Committee Report, 2009).

Owing in large part to the inconvenience of measuring fasting plasma glucose levels or performing an OGTT, and day-to-day variability in glucose, an alternative to glucose measurements for the diagnosis of diabetes has long been sought. HbA1c has now been recommended by an International Committee and by the ADA as a means to diagnose diabetes (International Expert Committee Report, 2009).

In 2009, The International Expert Committee recommended the use of HbA1c to diagnose diabetes mellitus with a threshold > 6.5%.1 The American Diabetes Association (ADA) adopted this recommendation in its position statement entitled, "Standard of medical care in diabetes-2010" (American Diabetes Association, 2010). However, the diagnosis should be confirmed by a repeat test unless symptoms of hyperglycaemia and blood glucose level of >11.1 mmol/l (>200mg/dl) are available. The diagnostic test should be standardized to Diabetes Control and Complication Trial (DCCT) reference assay or the National Glycohaemoglobin Standardisation Programme (NGSP) certified method (American Diabetes Association, 2009).

In addition, those with an HbA1c level between 6 and 6.5% have been identified as being at very high risk of developing diabetes. Prospective studies indicate that a high normal HbA1c level of 5.5% to 6.5% poses very high risk for subsequent development of diabetes, and the risk increases substantially as the values increase. (Sato *et al*., 2009; Edelman *et al*., 2004)

The ADA's recommended goal for HbA1c is < 7% in all patients with diabetes mellitus. The same level is recommended for primary prevention of cardiovascular disease in people with diabetes. The ideal HbA1c goal for individual diabetic patients is as low as <6% without causing significant hypoglycaemia. The recommendations are the same for T1DM and T2DM. The ADA recommends checking HbA1c levels at least twice a year in patients with relatively stable glycaemic control and quarterly among those whose HbA1c targets are not achieved, particularly if drugs are changed, to determine the effect of such changes. (American Diabetes Association, 2010).

2.5.1 HbA1c and CHD in Patients with Diabetes:

Much published data support the conclusion that diabetes puts people at very high risk of coronary heart disease (Folson *et al*., 2003). Diabetic patients without prior myocardial infarction have high risk of having myocardial infarction at rates comparable to non-diabetic patients with previous myocardial infarction (Haffner *et al*., 1998). Diabetes patients face an 11% increased risk of mortality from ischaemic heart disease (UKPDS 23) (Turner et al., 1998). While those with HbA1c > 8% face a 150% increased risk of death from heart disease (Saydah *et al*., 2009). This association is so well established that the Adult Treatment Panel III (NCEP) recommended treating diabetics with hyperlipidaemia as if they already have prior coronary artery disease (NCEP, 2002).

Cardiovascular complications are usually present at the diagnosis of T2DM, because diabetes is preceded by long period of asymptomatic hyperglycaemia, called impaired glucose tolerance. Classical CV risk factors such as smoking, hypertension, and hypercholesterolemia do not account for the excess risk of CV morbidity and mortality in patients with elevated HbA1c levels.15 This association is equally important in both T1DM and T2DM, and exists across ethnic and geographical boundaries. In another very large multiethnic population study (n= 47,904), HbA1c levels were shown to be strongly associated with increased all-cause mortality (Selvin *et al*., 2005).

2.5.2 HbA1c and CHD in Non-diabetic Population:

As previously noted, HbA1c levels below the threshold for a diagnosis of diabetes (< 6.5%) are associated with a very high risk of CHD: such patients should receive demonstrably effective preventive treatment **(**American Diabetes Association, 2010**).** In a study by E. Selvin *et al*, after 15 years followup of more than 11000 participants, suggested that HbA1c values in normal range without diabetes can identify people at higher risk of CAD, stroke and death (Selvin *et al*., 2010).

In the non-diabetic population (in women), HbA1c is a better predictor of CVD and CHD related mortality than fasting or post prandial glucose levels (Park *et al*., 1996). Elevated glycated haemoglobin is associated with increased cardiovascular mortality in non-diabetic chronic kidney disease (Menon *et al*., 2005). Every 1% increase in HbA1c is associated with a 20 to 30% increase in cardiovascular events, and all cause mortality in men and women independent of diabetic status (Khaw *et al*., 2004). Elevated HbA1c levels have also been associated with increased short-term mortality in post myocardial infarction, non-diabetic patients (Chowdhury and Lasker, 1998).

HbA1c showed a continuous relationship with increased CV mortality and extended to non-diabetic subjects (Khaw *et al*., 2004). This risk factor indicates excess mortality across the entire population distribution, even at moderately elevated levels (Khaw *et al*., 2001). In another epidemiological study of Asian Indian population, a strong association was found between HbA1c and other risk factors of metabolic syndrome including high fasting insulin and insulin resistance, independent of age and sex. The findings of the study become increasingly significant when the study population includes people with normal glucose tolerance (Dilley *et al*., 2007).

Although much published data proved the relationship of elevated HbA1c and incidence of coronary artery disease, intervention studies were needed to demonstrate that lowering HbA1c would reduce the risk of cardiovascular morbidity and mortality. Two land mark trials namely, the United Kingdom Prospective Diabetes Study (UKPDS) in T2DM and the Diabetes Control and Complication Trial (DCCT) in T1DM fulfilled the need. UKPDS-33 demonstrated that lowering HbA1c would result in a 10% lower incidence of diabetes related deaths (death from myocardial infarction, stroke, peripheral arterial disease, and sudden death) (UKPDS, 1998).

UKPDS-35 demonstrated a 21% reduction in the risk of diabetes related deaths and 14% reduction in the incidence of myocardial infarction with only a 1% reduction in HbA1c in T2DM (Stratton et al., 2000). Similarly, in T1DM, DCCT demonstrated intensive treatment delays the onset and progression of retinopathy, nephropathy and neuropathy and also the macro-vascular disease (DCCT, 1995). The DCCT patient population was relatively younger and, therefore, did not have a high incidence of cardiovascular events, which was probably the reason for very trivial reduction in the risk of CVD. However, when the same population sample was followed up for another 12 years in Epidemiology of Diabetes Intervention and Complication trial (EDIC), HbA1c reduction resulted in a 42% reduction in risk of CVD and 57% reduction in nonfatal heart attack, stroke, or death from cardiovascular causes (DCCT/ EDIC, 2005)

2.5.3 Glycated Hb and Heart Failure:

Elevated levels of glycated Hb not only increases incidence of CHD but also extends into subsequent complications of CHD (e.g., congestive heart failure); each 1% rise in HbA1c results in more coronary events and more hospitalizations due to worsening

heart failure (Gerstein *et al*., 2008). Elevated HbA1c (6.5% to 7%) is associated with poorer prognosis in patients with minimal left ventricular dysfunction (LVEF < 45%) (Goode *et al*., 2009).

This increased risk of developing heart failure could be explained by two mechanisms: the development of endothelial dysfunction, atherosclerosis, and CHD, and direct toxic damage to the myocardium due to chronic hyperglycaemia (Iribarren *et al*., 2001). Among diabetics, HbA1c is an independent predictor of heart failure even without pre-existing CHD, although the relative incidence rate of heart failure in people with pre-existing CHD is much higher (15.5 for CHD-negative vs. 56.4 for

CHD-positive cases per 1000 person) (Pazin- Filho *et al*., 2008).

When incidence of heart failure was compared between diabetic and non-diabetic populations, the incidence was 2.5 times higher among diabetic patients with elevated HbA1c (Nicholas *et al*., 2004). The difference in incidence heart failure was much higher among the younger age group, emphasizing the need for a good glycaemic control just after diagnosis.

2.5.4 Glycated Hb and Cerebrovascular Disease:

Patients of diabetes develop increasing intimal media thickness quicker than nondiabetics. Hard carotid artery plaques develop with increasing level of HbA1c, and risk increases continuously across increasing HbA1c levels (Jorgensen *et al*., 2004). Diabetes particularly puts younger people (<55 years) at risk of stroke and is a cause of poor prognosis post stroke. Raised HbA1c is an independent risk of stroke in people with or without diabetes, unlike CHD this relationship is not linear but it seems more like a threshold dependent association, risk of stroke abruptly increases at HbA1c >

7% regardless of diagnosis of diabetes. Although the people with HbA1c 6.5% - 7% are more likely to be undiagnosed diabetics, show significantly increased risk of stroke after adjustment for classic risk factors like hypertension and smoking (Myint *et al*., 2007)

A large meta-analysis of 33040 patients; however showed only non significant reduction in the event of stroke (7%) comparing to quite significant reduction in nonfatal MIs (17%) by only 0.9% reduction in HbA1c concentration after 5 years of treatment (Myint *et al*., 2007)

2.5.5 Glycated Hb and Peripheral Vascular Disease:

Diabetes increases risk of PVD by more than two folds, and is cause of 70% nontraumatic amputations. It is a manifestation of CVD which is not acutely fatal but is associated with serious risk of CHD and stroke. HbA1c is positively associated with low ankle-brachial index and symptomatic PVD (Selvin *et al*, 2006), although it is not only due to macrovascular disease but a great element of microvascular disease is also present in processes like neuropathy, foot ulceration, and amputations.

2.5.6 Probable Reasons of Misleading HbA1c Results

Glycated haemoglobin is formed by non-enzymatic glycation of N-terminus of beta chain of haemoglobin. Different Laboratory techniques and many clinical conditions may result in under estimation or over-estimation of HbA1c. Clinicians should interpret very low (<4%) or very high (15%) results with caution and concurrent diseases should be kept in mind. Falsely low HbA1c is seen mainly in conditions with

high red cell turn over, such as haemoglobinopathies including variant haemoglobins, sickle cell disease, glucose-6-phosphate dehydrogenase deficiency, treatment of anaemia with iron or erythropoietin, and auto immune haemolytic anaemia. Recent blood loss and blood transfusion result in greater proportion of reticulocytes or transfused red cells in blood stream thereby reducing the average age of red cells. Patients of chronic kidney disease on dialysis and chronic liver failure may also have less than expected level of HbA1c (Khaw *et al.*, 2004).

All these conditions result in shortened average age of erythrocytes, resulting in decreased exposure time of haemoglobin to glucose and therefore less percentage of HbA1c. Falsely low levels of HbA1c are also observed because glycated Hb variant is separated from HbA1c so it is excluded from calculations. Anaemia of iron, folic acid, and vitamin B12 deficiency could result in falsely high levels of HbA1c. Haemoglobin variants, eg, Hb Raleigh, Hb Graz and persistence of foetal haemoglobin or rise of HbF during pregnancy may also yield falsely high HbA1c levels. The possible explanation is that HbF is separated from HbA therefore proportion of HbA1c increases. Urea reacts with haemoglobin molecules at the same site as does glucose, therefore HbA1c and Car Hb have same isoelectric point and assayed together and results in falsely high percentage of HbA1c in uraemic patients (Khaw *et al.*, 2004).

2.6 ANTHROPOMETRY IN TYPE 2 DIABETES

Type 2 diabetes is commonly associated with obesity, hypertension, CVD, and lipid abnormalities. Approximately 58% of diabetes and 21% of ischemic heart disease globally are attributable to a body mass index (BMI) above 21 kg/m^2. At the same time, around 60% of low income patients borrow, mortgage, or sell their property just to keep their blood sugar levels under control (World Health Organization, 2002).

Anthropometric parameters are commonly used as research tools to assess the non communicable disease risk factors in the populations as they are inexpensive and easy to monitor at the community level (Elderman *et al*., 2004). Over a period of time these anthropometric parameters have evolved into reliable indicators for predicting the incidence of various noncommunicable disease risk factors in all populations though the threshold cut off values vary from population to population.

Various studies have shown that anthropometric parameters such as BMI, waist circumference (WC), waist hip ratio (WHR), and waist height ratio (WHtR) are useful indicators for predicting incidence of type 2 diabetes in populations (Stevens *et al*., 2001; Lincoln *et al*., 2002).

2.6.1 Body Mass Index

Body Mass Index is a number calculated from a person's weight and height. BMI is a fairly reliable indicator of body fatness for most people. Even though BMI does not measure body fat directly, research has shown that the index correlates to direct measures of body fat such as under water weighing and dual energy x-ray

absorptiometry (DEXA).However,the correlation varies by sex, race, and age (Donoghue,1985).These variations include the following examples:

- At the same BMI, women tend to have more body fat than men.
- At the same BMI, older people, on average, tend to have more body fat than younger adults.

The advantage of BMI is that it is an inexpensive and easy-to-perform method of screening for weight categories that may lead to health problems.

By WHO criteria, based on the international classification of adults, a person with a BMI between 18.5 and 25 kg/m2 is considered as healthy. A person with a BMI over 25 kg/m2 but less than 30kg/m2 is considered overweight and a person with BMI over 30kg/m2 is considered obese.

BMI can be underestimated in situations where individuals have lost muscle mass - this often occurs with older people and people with a physical disability who are unable to walk and may have muscle wasting. For people who are shorter (for example Asian populations), the cut-offs for overweight and obesity may need to be lower. This is because there is an increased risk of diabetes and cardiovascular disease, which begins at a BMI as low as 23 in Asian populations. Finally, BMI is not an accurate indicator for people with eating disorders like anorexia nervosa or people with extreme obesity (Donoghue, 1985).

2.6.2 Diseases Associated With Obesity and High BMI

In general, the greater the BMI the greater the susceptibility to diseases associated with obesity. These diseases include:

- High Blood Pressure: This can lead to heart failure, stroke, kidney damage and or loss of vision due to retinal damage.
- Diabetes - a metabolic disorder which can be caused by insulin resistance.
- Arteriosclerosis - narrowing & thickening of the arteries which can cause cerebrovascular and cardiovascular disorders.
- Hyperlipidaemia - a high level of fat in the blood which is associated with high cholesterol levels.

2.6.3 Waist Circumference, Waist Hip Ratio and Associated Health Risks

Abdominal obesity is increasingly being recognized as a major risk factor for cardiovascular disease. Compared with body mass index, waist circumference (Waist circumference is the measure of the waist specifically at the upper hip bone with a tape measure) and waist-to-hip ratio (This ratio is calculated by dividing the hip circumference by waist circumference at its narrowest point) appear to be more strongly associated with metabolic risk factors, incident CVD events, and death. The cardiovascular and metabolic risks associated with abdominal obesity are attributed to the presence of visceral adipose tissue (VAT), which promotes insulin resistance, dyslipidaemia, and hypertension. VAT stores can be measured by computerized axial tomography, magnetic resonance imaging, and dual energy x-ray absorptiometry, but these techniques are not feasible and too expensive for everyday use.

WC and WHR are the most common proxy measures of VAT. Both measures are correlated with VAT even though WC is more strongly associated with VAT. Despite this, WHR may be a better predictor of CVD risk as hip circumference is inversely associated with the development of cardiovascular and metabolic risk factors and

CVD. In their analysis of waist circumference and waist to hip ratio as predictors of cardiovascular events, Koning et al., (2007) noted that abdominal obesity as measured by WC and WHR is significantly associated with the risk of incident CVD events. They established that a 1 cm increase in WC is associated with a 2% increase in risk of future CVD and a 0.01 increase in WHR is associated with a 5% increase in risk of developing CVD.

2.6.4 Percentage Body Fat

A person's body is composed of major components such as bone, muscle, organ and fat. The amount or percentage of the fat component is of great interest to many people as it has correlation with health (Donoghue, 1985). A considerably high percentage of body fat especially when concentrated at the visceral section of the body predisposes one to cardiac disorders, type II diabetes and hypertension. Unfortunately, people cannot tell by simply weighing themselves and looking at BMI chart what their percentage body fat is or if they are over fat or under fat. This is because the chart makes no allowance for muscle development, and most people don't really know what their frame size is (Donoghue, 1985).

Chapter 3

METHODOLOGY

3.0 STUDY AREA AND SETTING

The study covered the period of October 2014 to June 2015 in the Eastern Regional Hospital, Ghana. This was carried out with some assistance from the Laboratory Department of Eastern Regional Hospital, Koforidua. All procedures were approved by the Committee on Human Research Publication and Ethics of School of Medical Sciences, KNUST

3.1 STUDY DESIGN

A cross sectional study of 200 type 2 diabetic patients. The participants were already diagnosed as type 2 diabetics and undergoing treatment.

A written informed consent form was completed by all the participants who were recruited into the study. The purpose of the study was explained to the participants in English and / or local language they understand. Pre-tested questionnaires were used

to record information of the participants. Information on demography, life style and anthropometric measurements were taken.

3.2 SELECTION OF PARTICIPANTS

A total of 200 type 2 diabetic patients attending the diabetic clinic at the Eastern Regional Hospital, Koforidua were randomly selected for the study. The participants were already diagnosed as having type 2 diabetes and were under treatment at the diabetic clinic.

3.2.1 Inclusion Criteria

Subjects already diagnosed as type 2 diabetic and under treatment attending the Diabetic clinic were included in the study.

3.2.2 Exclusion Criteria

Newly diagnosed diabetic patients who are not on treatment and non diabetics were excluded from the study. Smokers, alcoholics, and subjects with hepatic, renal, endocrine disorders, and those on lipid lowering agents were excluded from the study. These were verified by first checking from their medical records and through the use of a questionnaire.

3.3. MEASUREMENT OF ANTHROPOMETRY AND BLOOD PRESSURE

3.3.1 Weight Measurements

Weights of the participants were measured to the nearest 0.1kg. The scale was placed on a hard surface. The participants were then asked to wear light garment and stood in the center of the platform bare footed with their weight distributed evenly to both

feet.

3.3.2 Height Measurement

Heights of participants were measured to the nearest 0.5cm using wall mounted standiometer. Participants were asked to remove their sandals and made to stand upright with their back to the height rule.

3.3.3 Waist Circumference

Waist circumference of the participants was measured at a level midway between the lower rib margin and iliac crest with the tape all around the body in horizontal position. Participants were made to stand with their feet fairly close together and their weight equally distributed to each leg.

3.3.4 Hip Circumference

Hip circumference was measured as the maximal circumference over the buttocks.

3.3.5 Body Mass Index (BMI)

BMI was calculated as weight kg/height squared (kg/m^2) and subjects were considered as normal weight if their BMI was < 25 kg/m^2 , overweight if their BMI was from 25 to 29 kg/m^2 and obese if their BMI was ≥ 30 kg/m^2.

3.3.6 Body Adiposity Index (BAI)

Body Adiposity Indices was calculated using the formula

$$\text{BAI} = \frac{\text{hip in cm}}{(\text{height in m})^{1.5}} - 18$$

3.3.7 Visceral Adiposity Index (VAI)

Visceral Adiposity Indices was calculated using the formula

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

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

3.3.8 Blood Pressure

Mercury sphygmomanometer and stethoscope were used to measure the blood pressure after the patient has been allowed to rest for at least 10 minute. To ensure accurate readings, an appropriate-size blood pressure cuff was used and blood pressure of each patient was taken twice at one minute interval and the average recorded. Those whose average systolic blood pressure (SBP) was ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg were defined as hypertensive.

3.4. ASSAY OF HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS

About 6ml of fasting blood samples (overnight fast between 8-12 hours) were drawn from the median cubital vein on the anterior forearm (the side within the fold of the elbow) into clot activator/ separating gel tubes (to aid clotting and separate serum), fluoride oxalate tubes (to prevent glycolysis) and EDTA BD vacutainer®, (BD, Plymouth, PL6 7BP. UK).

The clotted blood was centrifuged (Zentrifugen, D-78532, Tuttlingen, Germany) at 2000 rpm for 5 minutes to separate the serum from the deposit. The serum was used to estimate the lipid profile: Total cholesterol, High density lipoprotein cholesterol and low density lipoprotein. Remaining serum which were not immediately used were stored at -20^{o}C.

The EDTA anticoagulated blood was gently mixed with blood mixer (Sarstedt, D-5223, Numbrecht, West Germany). The whole blood was used for Glycated haemoglobin concentration, Haemoglobin concentration, RBC count and Haemoglobin electrophoresis.

The fluoridated anticoagulated blood was centrifuged (Zentrifugen, D-78532, Tuttlingen, Germany) at 3000 rpm for 5 minutes to separate the plasma from the deposit. The plasma was used to estimate the fasting blood glucose. All analysers were calibrated before the start of the analyses. Results which were flagged as high or low were repeated to verify their reproducibility.

For serum lipid reference level, National Cholesterol Education Programme (NCEP) Adult Treatment Panel III (ATP III) guideline was referred. According to NCEPATPIII guideline, hypercholesterolemia is defined as TC>5.2 mmol/l, high LDL-C when value >2.6 mmol/l, hypertriglyceridemia as TG >3.8 mmol/l and low HDL-C when value <1.0 mmol/l. Dyslipidemia was defined by presence of one or more than one abnormal serum lipid concentration. Diabetes was defined as per American Diabetes Association (ADA) criteria.

3.4.1 REACTION PRINCIPLES

3.4.1.1 Glycated Haemoglobin (HBAIC) (Immunoturbidimetric Method)

Glycated Haemoglobin was determined using BT 3000 Plus auto analyzer (Biotecnica Instruments S.p.A.Via Licenza, Rome – Italy). The method is based on a combination of the latex-enhanced competitive turbidimetric immunoassay for HbA1c and colorimetric determination of total hemoglobin within the same sample. The first step

in the immunoassay is enzymatic cleavage of hemoglobin from lysed erythrocytes, whereby b -N-terminal fragments are released for reaction with a fixed amount of latex-bound monoclonal antibodies. The monoclonal antibodies for this assay is raised against a synthetic peptide consisting of N-terminal hexapeptide identical to human hemoglobin b -chain and glycated on N-terminal valine residue, which is structurally analogous to HbA1c. Antibody excess is then removed by agglutination with a synthetic polyvalent complex of b -N-terminal fragments.

The reaction is monitored by turbidimetry with light-scattering signal (absorbance at 540 nm) inversely related to the amount of HbA1c in the sample. Total hemoglobin is measured in the same hemolysate by the alkaline hematin method (absorbance at 410 nm). The final HbA1c result (expressed as percentage) is then calculated from the HbA1c/tHb ratio.

3.4.1.2 Fasting Plasma Glucose (GOD-PAP Method)

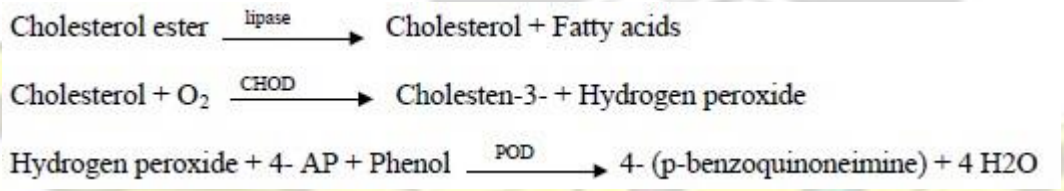
Fasting plasma glucose was determined using BT 3000 Plus auto analyzer (Biotecnica Instruments S.p.A.Via Licenza, Rome – Italy). 10µ of plasma was aspirated by the auto analyzer with internal temperature of 37OC and an incubation period of 10 minutes. Glucose was determined after enzymatic oxidation in the presence of glucose oxidase (EC 1.1.3.4) at 37 ^{O}C for 10minutes. The formed hydrogen peroxide reacts under catalysis of peroxidase, POD (EC 1.11.1.7) with phenol and 4-aminophenazone to redviolet quinoemine dye as indicator. This was measured at a wavelength of 505nm.

$$\text{Glucose} + O_2 + H_2 \xrightarrow{\text{glucose oxidase}} \text{gluconic acid} + H_2O_2$$

$$2H_2O_2 + 4\text{AP} + \text{phenol} \xrightarrow{\text{POD}} 4H_2O + 4\ (\text{p-benzoquinoneimine})\ \text{phenazone}$$

3.4.1.3 Total Cholesterol (GOD-PAP Method)

Total cholesterol was determined by using BT 3000 Plus auto analyzer (Biotecnica Instruments S.p.A.Via Licenza, Rome – Italy). 10µl of plasma was aspirated by the auto analyzer with internal temperature of 37^{O}C and an incubation period of 10 minutes. In this method, Cholesterol is enzymatically oxidized by cholesterol esterase (CHOD) (EC1.1.3.6) after hydrolysis of its esters with a fungal lipase. Hydrogen peroxide released produces the oxidative coupling of phenol with 4-aminophenazone (4AP) by means of a reaction catalysed by peroxidase (POD) (EC 1.11.1.7) yielding quinoneimine which is read at 505nm.



3.4.1.4 Triglycerides (GOD-PAP Method)

Triglycerides were determined enzymatically with BT 3000 Plus auto analyzer (Biotecnica Instruments S.p.A.Via Licenza, Rome – Italy). 10ul of plasma was aspirated by the auto analyzer with internal temperature of 37OC and an incubation period of 10 minutes. In this method, the triglycerides were determined after enzymatic hydrolysis with lipases. Indicator is quinoneimine formed hydrogen peroxide, 4-aminoantipyrine and 4-chlorophenol under the catalytic influence of peroxidase.

$$TGs + H_2O \xrightarrow{LPL} Glycerol + FAs$$

$$Glycerol + ATP \xrightarrow{GK} Glycerol\text{-}3\text{-}p + ADP$$

$$Glycerol\text{-}3\text{-}p + O_2 \xrightarrow{GPO} Dihydroxyacetone\ phosphate + H_2O_2$$

$$2\,H_2O_2 + 4\text{-}aminophenazone + 4\ Chlorophenol \xrightarrow{POD} quinoneimine + HCl + 4H_2O$$

3.4.1.5 High Density Lipoprotein Cholesterol

HDL cholesterol was determined using BT 3000 Plus auto analyzer (Biotecnica Instruments S.p.A.Via Licenza, Rome – Italy). The method was by direct homogenous test. In this method, the assay combines two steps. In the first step chylomicrons, VLDL and LDL cholesterol are specifically eliminated and destroyed by enzymatic reactions. In the second step, remaining cholesterol formed from the HDL fraction is determined by well established specific enzymatic reactions in the presence of surfactants for the HDL.

First Step:

$$LDL,\ VLDL,\ Chylomicrons \xrightarrow{(CHE+CHO)} Cholestenone + H_2O_2$$

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

Second Step:

$$HDL \xrightarrow{(CHE+CHO)} Cholestenone + H_2O_2$$

$$H_2O_2 + Chromogen \xrightarrow{(Peroxidase)} quinine\ pigment$$

3.4.1.6 Low Density Lipoprotein

LDL cholesterol was determined enzymatically with BT 3000 Plus auto analyzer (Biotecnica Instruments S.p.A.Via Licenza, Rome – Italy). The method was by direct homogeneous test. In this method, the assay combines two steps. In the first step chylomicrons, VLDL and HDL cholesterol are specifically removed by enzymatic

reactions. In the second step remaining LDL cholesterol is determined by enzymatic reactions, employing specific surfactants for LDL.

First Step

HDL, VLDL, Chylomicrons $\xrightarrow{(CHE+CHO)}$ Cholestenone + H_2O_2

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

Second Step

LDL $\xrightarrow{(CHE+CHO)}$ Cholestenone + H_2O_2

H_2O_2 + Chromogen $\xrightarrow{(Peroxidase)}$ quinine dye

3.4.1.7 Determination of Haemoglobin Concentration and Red Blood Cell Count (Sysmex XT 2000i Haematology Analyzer)

Venous blood sample was collected aseptically from each subject into a tube containing tri-potassium ethylenediamine tetra-acetic acid (K_3EDTA) for the analysis of Haemoglobin concentration and Red Blood Cell Count using Sysmex (XT-2000i). The whole Blood was gently but thoroughly mixed and 10ul aspirated by the haematology analyzer to determine the Hb and RBC count.

3.4.1.7.1 Sodium Lauryl Sulfate (SLS) Hemoglobin Analysis Method

The SLS-hemoglobin method is an analysis method that makes use of the advantages of two methods; cyanmethaemoglobin and oxyhaemoglobin. In the SLS-Haemoglobin method, surfactants lyse the red blood cell membrane releasing haemoglobin. The globin group of the haemoglobin molecule is altered by the hydrophilic alkyl group of sodium lauryl sulfate. This induces the conversion of haemoglobin from the ferrous (Fe+2) to the ferric (Fe+3) state forming methaemoglobin, which combines with sodium lauryl sulfate to become SLS-Hb haemichrome molecule. Light (of wavelength

555nm) emitted from the diode passes through the sample in the haemoglobin cell of the analyzer. The concentration of SLS- Haemoglobin is analysed as light absorbance.

3.4.1.7.2 Test Method for Red blood cell count (Flow Cytometry Method Using Semiconductor Laser)

A blood sample is aspirated, measured, diluted to the specified ratio, and stained. The sample is then fed into the flow cell. This sheath flow mechanism improves cell count accuracy and reproducibility. Since the blood cell particles pass in a line through the center of the flow cell, the generation of abnormal blood pulses is prevented and flow cell contamination is reduced. A semiconductor laser beam is emitted to the blood cells passing through the flow cell. The forward scattered light is received by the photodiode, and the lateral scattered light and lateral fluorescent light are received by the photo multiplier tube. This light is converted into electrical pulses, thus making it possible to obtain blood cell information. The result obtained is then printed out.

3.4.1.8 Haemoglobin Electrophoresis (Alkaline cellulose acetate method)

Haemoglobin electrophoresis is used to separate and identify the different haemoglobins by their migration within an electric field. Haemoglobin variants separate at different rates due to differences in their surface electrical charge as determined by their amino acid structure. An alkaline buffer at Ph 8.4–8.6 using a cellulose acetate membrane provides the medium for separation. This method is in two phases: haemolysate preparation and the test method.

Equipment and materials used:

1. Electrophoretic tank
2. Power supply of 350V, 50A
3. Cellulose acetate paper

4. EDTA Borate buffer, pH 8.6
5. Lyzing reagent, (3.8g EDTA and 0.7 g potassium cyanide dissolved in 1 litre of distilled water.)
6. Applicator

Haemolysate preparation:

1. Whole blood sample in EDTA was first centrifuged to remove the plasma.
2. The red cells were then washed three times in physiological saline. After each wash, it was then centrifuged at about 1000g for 5 minutes. The saline was then removed.
3. The red cells was then lysed with 1.5 volumes distilled (deionized) water and 1 volume of toluene. It was then well- shaken several minutes in a stoppered tube and then finally centrifuged at about 1000g for 20 minutes.

Test method:

1. Prepare the cellulose acetate membrane
2. 100 ml of the Tris-EDTA-borate buffer was poured into each of the outer sections of the Zip-Zone electrophoresis chamber.
3. 5 ul of each haemolysate sample (tests and controls) was transferred into the Zip-Zone well plate.
4. Cellulose acetate membrane (plate)was placed in the Zip-Zone aligning plate and sample was applied using an applicator
5. The cellulose acetate membrane (plate) was immediately placed in the electrophoresis chamber.
6. The chamber was connected to the Power Supply and the plate was

electrophoresed for 25 minutes (or shorter) at 350 volts and 50 mA.

7. The relative mobilities of the haemoglobins were then read using control samples of known haemoglobins.

3.5 STATISTICAL ANALYSES

The results were expressed as Mean ± SEM (Standard Error of the mean) instead of Standard Deviation which compares population. Statistical significant level was put at $P < 0.05$ unless otherwise stated. SPSS statistical package version 20 was used for data analysis. Comparison between the investigated anthropometric, clinical and laboratory parameters were established using Pearson's correlation coefficient (r). Independent samples t-test (2-tailed) was used to compare means of different parameters. Correlation was significant at the 0.05, 0.01, 0.001 levels (2 tailed).

Chapter 4

RESULTS

4.1 DEMOGRAPHICS, ANTHROPOMETRIC AND CLINICAL PARAMETERS OF THE STUDY POPULATION STRATIFIED BY GENDER

In all, 200 subjects were recruited for the study of which 53 (26.5%) were males and 147(73.5%) were females. The mean age of the males (58.1±2.3) were not statistically different (p=0.6402) from that of the females (57.1±1). The systolic blood pressure of the male subjects (140.1±3.3) and the female subjects (137.1±1.4) were not statistically different (p=0.3303). The same could be said of the diastolic blood pressure of the male and female subjects (p= 0.8178).

Some anthropometric variables between male and female subjects showed significant differences. The mean waist circumference (WC), hip circumference (HC), Body Adiposity Index (BAI) and Body Mass Index (BAI) of the female subjects were significantly high as compared with the male subjects (p=0.0008, 0.0002, 0.000 and 0.0002 respectively). The mean Visceral Adiposity Index (VAI) and weight (WT) of the male and female subjects were not different statistically (p= 0.1742 and 0.8048 respectively)

There were no statistically difference recorded in the biochemical variables among the male and female subjects with the exception of triglycerides (p=0.0059) which was higher in females than males. Table 4.1 shows the demographic, anthropometric and clinical variables among the male and female subjects of the study population.

Table 4.1 *Demographics, Anthropometric and clinical variables of the study population stratified by Gender*

PARAMETERS	CASES N=200	MALE CASES N=53	FEMALE CASES N=147	P-VALUE
AGE (Years)	57.4±1	58.1±2.3	57.1±1	0.6402
Clinical variables				
DUR (Years)	6.2±0.3	4.9±0.5	6.7±0.3	**0.0036**
SBP (mmHg)	137.9±1.4	140.1±3.3	137.1±1.4	0.3303
DBP (mmHg)	82.0±0.8	81.7±1.6	82.1±0.9	0.8178
Anthropometry				
WT (Kg)	69.6±1	70.0±1.4	69.4±1.3	0.8048
HT (cm)	160.4±0.6	167.7±0.9	157.8±0.6	**0.0012**
WTCIR (cm)	96.6±1.1	90.6±1.7	98.7±1.3	**0.0008**
HIPCIR (cm)	100.3±0.9	94.8±1.7	102.2±1	**0.0002**
WAIST/HIP RATIO	1.0±0	1.0±0	1.0±0	0.6311
VAI	4.1±0.8	2.2±0.2	4.8±1.1	0.1742
BAI	31.5±0.5	25.7±0.8	33.6±0.5	**0.0008**
BMI (kg/m²)	27.1±0.4	24.9±0.4	27.8±0.5	**0.0002**
Biochemical				
TCHOL (mmol/l)	5.8±0.1	5.6±0.3	5.9±0.1	0.2287
TRIG (mmol/l)	1.7±0.1	1.4±0.1	1.8±0.1	**0.0059**
HDL-C (mmol/l)	1.4±0.1	1.4±0.2	1.3±0.1	0.5248
LDL-C (mmol/l)	3.7±0.1	3.6±0.2	3.8±0.1	0.3821
NON-HDL-C (mmol/l)	1.6±0.1	4.2±0.2	4.6±0.1	**0.0070**
TC/HDL	4.5±0.1	1.6±0.2	1.6±0.1	0.9420
LDL/HDL	3.5±0.1	3.6±0.3	3.5±0.2	0.6830

FBS (mmol/l)	9.6±0.3	8.7±0.5	9.9±0.4	0.1002
HBAIC (%)	8.7±0.1	8.3±0.3	8.9±0.2	0.1013
HB (g/dl)	12.1±0.4	12.9±0.1	11.8±0.5	0.1685
RBC count (x10^{12}/L)	4.3±0.2	4.6±0.1	4.2±0.2	0.3566

Values are presented as Mean ± standard error of mean (SEM). P<0.05 was considered statistically significant difference. Independent sample T-test was used to compare means of Demographics, Anthropometric and clinical variables of male and female cases. WT: Weight, HT: Height, DUR: Duration of diabetes, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, WTCIR: Waist circumference, HIPCIR: Hip circumference, VAI: Visceral Adiposity Index, BAI: Body Adiposity Index, BMI: Body Mass Index, TCHOL: Total cholesterol, TRIG: Triglycerides, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol, FBS: Fasting blood glucose, HBA1C: Glycated haemoglobin, HB: Haemoglobin, RBC: Red blood cell

4.2 DEMOGRAPHICS AND ANTHROPOMETRIC AND CLINICAL PARAMETERS OF THE STUDY POPULATION STRATIFIED BY HBA1C

Subjects with desirable HBAIC levels (HBA1C≤ 7.0) had a lower mean duration of diabetes (4.2±0.3) as compared with those of higher HBA1C levels (6.7±0.3) which is statistically significant (p= 0.001). Body Adiposity Index, Body Mass Index, LDL-C, Triglycerides and Fasting Blood Sugar levels were all significantly lower from those with higher HBA1C levels (p= 0.032, 0.027, 0.040, 0.042 and 0.001 respectively). The mean levels of HDL-C and Haemoglobin were significantly lower in subjects with higher HBA1C levels as compared with those of lower HBA1C.

On the other hand, mean systolic blood pressure, diastolic blood pressure, visceral adiposity index and total cholesterol were not statistically different for both subjects with desirable and high HBA1C levels (p= 0.279, 0.864, 0.068 and 0.604 respectively). Table 4.2 show the demographic, anthropometric and clinical variables of the study population stratified with HBAIC levels.

Table 4.2 *Demographics, Anthropometric and clinical variables of the study population stratified by Glycated Haemoglobin levels*

PARAMETER	**CASES N=38**	**HBA1C≤ 7.0 N=162**	**HBA1C>7.0**	**VALUE**	**P-N=200**
Demographic					
AGE (year)		57.4±1	56.0±2.6	57.4±1	0.229
Clinical variables					
DUR (year)		6.2±0.3	4.2±0.3	6.7±0.3	**0.001**
SBP		137.9±1.4	140.9±3.6	137.2±1.5	0.279
DBP		82.0±0.8	81.7±2.1	82.0±0.9	0.864
WT (Kg)		69.6±1	67.6±1.8	70.0±1.2	0.345
HT (cm)		160.4±0.6	162.9±1.3	159.9±0.7	0.052
WTCIR (cm)		96.6±1.1	95.8±2.3	96.7±1.2	0.732
HIPCIR (cm)		100.3±0.9	98.3±1.5	100.7±1	0.290
WAIST/HIP RATIO		1.0±0	1.0±0	1.0±0	0.327
VAI		4.1±0.8	7.2±4.4	3.3±0.2	0.068
BAI		31.5±0.5	29.4±0.8	32.1±0.6	**0.032**
BMI (Kg/m^2)		27.1±0.4	25.4±0.5	27.4±0.4	**0.027**

Biochemical				
TCHOL (mmol/l)	5.8±0.1	5.7±0.4	5.9±0.1	0.604
TRIG (mmol/l)	1.7±0.1	1.6±0.1	1.7±0.1	**0.040**
HDL-C (mmol/l)	1.4±0.1	1.6±0.2	1.3±0.1	**0.029**
LDL-C (mmol/l)	3.7±0.1	3.4±0.2	3.8±0.1	**0.042**
NON-HDL-C (mmol/l)	1.6±0.1	4.1±0.2	4.6±0.1	**0.049**
TC/HDL	4.5±0.1	1.5±0.2	1.6±0.1	0.072
LDL/HDL	3.5±0.1	3.0±0.3	3.7±0.2	0.683
FBS (mmol/l)	9.6±0.3	7.1±0.3	10.1±0.3	**0.001**
HB (g/dl)	12.1±0.4	13.7±1.9	11.7±0.1	0.030
RBC count (x10^{12}/L)	4.3±0.2	4.3±0.2	4.1±0.1	0.656

Values are presented as Mean ± standard error of mean (SEM). P<0.05 was considered statistically significant difference. Independent sample T-test was used to compare means of Demographics, Anthropometric and clinical variables of HBAIC categories. WT: Weight, HT: Height, DUR: Duration of diabetes, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, WTCIR: Waist circumference, HIPCIR: Hip circumference, VAI: Visceral Adiposity Index, BAI: Body Adiposity Index, BMI: Body Mass Index, TCHOL: Total cholesterol, TRIG: Triglycerides, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol, FBS: Fasting blood glucose, HBA1C: Glycated haemoglobin, HB: Haemoglobin, RBC: Red blood cell

4.3 CORRELATION BETWEEN HBA1C AND CARDIOVASCULAR RISK FACTORS

Glycated haemoglobin showed significant positive correlation with total cholesterol, triglycerides, LDL- cholesterol and fasting blood sugar. There were however a negative non-significant correlations with systolic blood pressure and a positive non correlation with diastolic blood pressure. Total cholesterol shows a significant positive correlation with triglycerides, HDL-cholesterol, LDL-cholesterol, fasting blood sugar,

systolic and diastolic blood pressure. Both triglycerides and HDL-cholesterol showed a significant positive correlation with fasting blood sugar. There was also a significant positive correlation between LDL-cholesterol and systolic and diastolic blood pressure. Glycated haemoglobin showed positive significant correlation with Body Adiposity Index but non-significant positive correlation with Body Mass Index and a negative correlation with Visceral Adiposity Index. BMI showed positive significant correlation between BAI. Duration of diabetes showed a positive significant correlation with glycated haemoglobin. Table 4.3 shows the correlation between HBA1C and cardiovascular risk factors.

TABLE 4.3 *Correlations between HBA1C and Cardiovascular Risk Factors*

	HBAIC	SBP	DBP	TC	TG	HDL	LDL	FBS	VAI	BAI	BMI	DUR	NON HDL
HBAIC (%)	1	-.045	.125	.181*	.011*	.052	.200**	.543**	-.093	.094	.001	.229**	.178*
SBP		1	.549**	.292**	.085	.137	.256**	-.085	-.022	.157*	.147*	.143*	.264**
DBP			1	.329**	.013	.180*	.300**	.129	-.028	.116	.096	.141*	.281**
TC (mmol/l)				1	.284**	.523**	.831**	.220**	-.053	.059	.037	.033	.872**
TG (mmol/l)					1	.181*	.003	.186**	-.004	.192**	.185**	.041	.236**
HDL-C (mmol/l)						1	.023	.343**	-.088	-.057	-.115	-.023	.039
LDL-C (mmol/l)							1	.023	-.016	.051	.062	.080	.960**
FBS (mmol/l)								1	-.078	-.144*	-.192**	.173*	.059
VAI									1	.019	-.013	.004	-.010
BAI										1	.671**	.050	.106
BMI											1	.011	.116
DUR (year)												1	.054
NHDL (mmol/l)													1

*. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed). SBP: Systolic blood pressure, DBP: Diastolic blood pressure, TCHOL: Total cholesterol, TRIG: Triglycerides, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol, FBS: Fasting blood glucose, HBA1C: Glycated haemoglobin, NHDL-C: Non High density lipoprotein cholester

4.4 COMPARISM OF BIOCHEMICAL PARAMETERS OF SUBJECTS WITH HIGH AND DESIRABLE HBA1C LEVELS

Table 4.4 *Comparism Of Biochemical Parameters Of Subjects With High And Desirable Hba1c Levels*

PARAMETER		HBA1C≤ 7.0%	HBA1C>7.0%	Chi²	P- value
TCHOL(mmol/l)	Normal	20(23.3%)	66(76.7%)	1.776	0.183
	High	18(15.8%)	96(84.2%)		
TG (mmol/l)	Desirable	23(18.5%)	101(81.5%)	0.043	0.835
	High	15(19.7%)	61(80.3%)		
HDL-C (mmol/l)	Low	26(17.4%)	123(82.6%)	0.913	0.339
	Desirable	12(23.5%)	39(76.5%)		
LDL-C (mmol/l)	Desirable	11(26.2%)	31(73.8%)	1.786	0.181
	High	27(17.1%)	131(82.9%)		
FBS (mmol/l)	Normal	21(34.4%)	40(65.6%)	13.571	0.0002
	High	17(12.2%)	122(87.8%)		
HB (g/dl)	Normal	20(17.2%)	96(82.8%)	0.555	0.456
	Anaemic	18(21.4%)	66(78.6%)		

Independent sample T-test was used to compare means of Biochemical Parameters of subjects with High and Desirable Hba1c Levels. DBP: Diastolic blood pressure,

TCHOL: Total cholesterol, TG: Triglycerides, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol, FBS: Fasting blood glucose, HB: Haemoglobin

4.5 COMPARISM OF ANTHROPOMETRIC, MEDICAL AND DEMOGRAPHIC PARAMETERS OF SUBJECTS WITH HIGH AND DESIRABLE HBA1C LEVELS

TABLE 4.5 *Comparism Of Anthropometric, Medical And Demographic Parameters Of Subjects With High And Desirable Hba1c Levels*

PARAMETER		HBA1C≤ 7.0%	HBA1C>7.0%	CHI Square	P value
DUR(year)	0-5	29(30.5%)	66(69.5%)	17.027	0.001
	6-10YEARS	9(11.0%)	73(89.0%)		
	11-15 YRS	0(0%)	17(100%)		
	16- 20 YRS	0(0%)	6(100%)		
SBP	Normal	20(20.2%)	79(79.8%)	0.184	0.668
	Hypertensive	18(17.8%)	83(82.2%)		
DBP	Normal	22(19.8%)	89(80.2%)	0.109	0.741
	Hypertensive	16(18.0%)	73(82.0%)		
WTHR	Normal	8(26.7%)	22(73.3%)	1.348	0.246
	Abnormal	30(17.6%)	140(82.4%)		
BMI(kg/m^2)	Normal	22(27.5%)	58(72.5%)	6.259	0.012
	Overweight	16(13.3%)	104(86.7%)		
BAI	Normal	22(20.2%)	87(79.8%)	0.218	0.641

	Abnormal	16(17.6%)	75(82.4%)		
VAI	Normal	13(18.8%)	56(81.2%)	0.002	0.967
	Abnormal	25(19.1%)	106(80.9%)		
AGE(year)	20-30	3(30.0%)	7(70.0%)	10.795	0.029
	31-40	1(10.0%)	9(90.0%)		
	41-50	9(40.9)	13(59.1%)		
	51-60	12(21.1%)	45(78.9%)		
	60+	13(12.9%)	88(87.1%)		

DBP: Diastolic blood pressure, TCHOL: Total cholesterol, TRIG: Triglycerides, HDLC: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol, FBS: Fasting blood glucose, HBA1C: Glycated haemoglobin, DUR: Duration of diabetes.

4.6 COMPARISM OF BIOCHEMICAL PARAMETERS STRATIFIED WITH DURATION OF DIABETES

TABLE 4.6 *Comparism of Biochemical parameters stratified with duration of diabetes*

PARAMETER		**0-5 YRS**	**6-10 YRS**	**11-15 YRS**	**15-20 YRS**	**Chi²**	**Pvalue**
FBS(mmol/l)	Normal	35(57.4%)	26(42.6%)	0(0%)	0(0%)	11.953	0.008
	High	60(43.2%)	56(40.3%)	17(12.2%)	6(4.3%)		
TCHOL(mmol/l)	Normal	42(48.8%)	37(43.0%)	3(3.5%)	4(4.7%)	6.037	0.11
	High	53(46.5%)	45(39.5%)	14(12.3%)	2(1.8%)		
TRIG(mmol/l)	Desirable	57(46.0%)	53(42.7%)	10(8.1%)	4(3.2%)	0.531	0.912

	High	38(50.0%)	29(38.2%)	7(9.2%)	2(2.6%)		
HDL(mmol/l)	Desirable	22(43.1%)	19(37.3%)	6(11.8%)	4(7.8%)	6.719	0.081
	Low	73(49.0%)	63(42.3%)	11(7.4%)	2(1.3%)		
LDL (mmol/l)	Desirable	18(42.9%)	20(47.6%)	1(2.4%)	3(7.1%)	6.193	0.103
	High	77(48.7%)	62(39.2%)	16(10.1%)	3(1.9%)		
HB (g/dl)	Normal	50(43.1%)	50(43.1%)	11(9.5%)	5(4.3%)	3.317	0.345
	Anaemic	36(43.1%)	36(43.1%)	8(9.5%)	6(4.3%)		
HBA1C (%)	Desirable	29(76.3%)	9(23.7%)	0(0.0%)	0(0.0%)	17.027	0.001
	High	66(40.7%)	73(45.1%)	17(10.5%)	6(3.7%)		

P<0.05 was considered statistically significant difference. TCHOL: Total cholesterol, TRIG: Triglycerides, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol, FBS: Fasting blood glucose, HBA1C: Glycated haemoglobin, HB: Haemoglobin, RBC: Red blood cell

4.7 DISLIPIDEMIA AMONG THE SUBJECTS

Hypercholesterolemia was found in 114(57%) individuals. Similarly, hypertriglyceridemia was found in 124(62%) individuals, decreased HDL-C was found in 149(74.5%) individuals and increased LDL-C was found in 158(79%) individuals. Among the diabetic individuals, 119(59.5%) individuals had only one abnormal lipid profile parameter, 68(34.0%) had two abnormal lipid parameter and 54(27%) individuals had more than 2 abnormal lipid profile parameter. Table 4.7 *Dislipidimia among the study subjects*

PARAMETER		FREQUENCY	PERCENTAGE
T CHOL (mmol/l)	Normal	86	43.0
	High	114	57.0
	Total	200	100.0
TRIG (mmol/l)	Desirable	124	62.0
	High	76	38.0
	Total	200	100.0
HDL (mmol/l)	Low	149	74.5
	Desirable	51	25.5
	Total	200	100.0
LDL (mmol/l)	Desirable	42	21.0
	High	158	79.0
	Total	200	100.0

TCHOL: Total cholesterol, TRIG: Triglycerides, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol, FBS: Fasting blood glucose

Chapter 5

DISCUSSION

In this study, 200 type 2 diabetic patients attending the diabetic clinic at the Eastern Regional Hospital, Koforidua were randomly selected for the study. The participants were already diagnosed as having type 2 diabetes and were under treatment at the diabetic clinic. The subjects included 143 females and 57 males. The female population was more than twice that of the male counterpart. This compares well with a study on WHO global data (WHO, 1998) which stated that the prevalence ratio of diabetes between men and women varies markedly, with no consistent trend.

It was generally inferred from the study that protein glycation occurs more higher among male subjects as strength of correlation was higher than their female

counterparts. The relative difference in frequency between the sexes is probably related to the presence of underlying factors, such as pregnancy and obesity, rather than to a sex-specific genetic tendency (American Diabetes Association, 2008). From this study, it was observed that the diabetic subjects were averagely older. This shows type 2 diabetes begins typically in middle life or later, the prevalence rises with age. This is consistent with studies published by WHO (1998). This also implies that impact of age as a risk factor of diabetes cannot be overemphasized as this trend has been demonstrated in most study populations around the world (Ford *et al,* 2002).

It was observed that duration of type 2 diabetes had a positive correlation with glycated haemoglobin (table 4.4). This is because the body becomes more resistant to insulin with increasing duration of diabetes. Various studies prove that the amount of carbohydrate attached to the HbA1c increases with increasing duration of the disease (Verma *et al*., 2006, Kilpatrick *et al*., 1996). In contrast Kabadi *et al* (1998) found no significant relation between age, duration of diabetes, fasting glucose and HbA1c. Abdominal obesity is increasingly being recognized as a major risk factor for cardiovascular disease. Compared with body mass index, waist circumference (Waist circumference is the measure of the waist specifically at the upper hip bone with a tape measure) and waist-to-hip ratio (This ratio is calculated by dividing the hip circumference by waist circumference at its narrowest point) appear to be more strongly associated with metabolic risk factors, incident CVD events, and death. The cardiovascular and metabolic risks associated with abdominal obesity are attributed to the presence of visceral adipose tissue (VAT), which promotes insulin resistance, dyslipidaemia, and hypertension.

In their analysis of waist circumference and waist to hip ratio as predictors of cardiovascular events, Koning *et al*., (2007) noted that abdominal obesity as measured by WC and WHR is significantly associated with the risk of incident CVD events. They established that a 1 cm increase in WC is associated with a 2% increase in risk of future CVD and a 0.01 increase in WHR is associated with a 5% increase in risk of developing CVD.

In the present study, it was noted that females had significantly higher BMI, BAI, hip circumference and waist circumference measurements than their male counterparts. The relative difference in frequency between the sexes is probably related to the presence of underlying factors, such as pregnancy and obesity, rather than to a sexspecific genetic tendency (American Diabetes Association, 2008). Subjects with higher HBA1C levels also had significantly higher measurements in BMI, BAI, waist circumference and hip circumference as compared with those of desirable HBA1C levels. Other studies (Patiakas et al., 2010 and Suhil *et al*., 2015) also established a relationship between HBA1C levels and BMI but found no correlation between HBA1C and BAI. Both studies even concluded that BAI is not a better indicator and cannot replace BMI in the study of anthropometric measurements in diabetic patients. From the study, there were no significant difference in the mean systolic and diastolic blood pressure of the male and female subjects. This was also the case among subjects with high and desirable HBA1C level groups. This finding is in agreement with studies conducted by Cabrales *et al*. (2008). However, studies conducted by Bower *at al*. (2012) and Patiakas *et al*. (2010) found a positive correlation between HBAIC and blood pressure measurements.

In this study, the pattern of lipid profile parameters in diabetic subjects and its correlation with HbA1c was evaluated. The levels of HbA1c and FBG did not differ significantly between male and female. Although there were no significant difference in TC (p=0.2287), LDL-C (p=0.3821) and HDL-C (p=0.5248) levels between male and female, the levels of TG were significantly higher (p=0.0059) in female as compared to male type 2 diabetic patients. This finding is in agreement with other studies (Ram *et al*., 2011, Masram *et al*., 2012). Hyperlipidemia in females may be attributed to the effects of sex hormones on body fat distribution, which leads to differences in altered lipoproteins (Sibley *et al*, 2006).

This study reveals high prevalence of hypercholesterolemia, hypertriglyceridemia, high LDL-C and low HDL-C levels among subjects with high HBA1C levels which are well known risk factors for cardiovascular diseases. Insulin affects the liver apolipoprotein production. It regulates the enzymatic activity of lipoprotein lipase (LpL) and Cholesterol ester transport protein. All these factors are likely cause of dyslipidemia in Diabetes mellitus (Goldsberg, 1996). Moreover, insulin deficiency reduces the activity of hepatic lipase and several steps in the production of biologically active LpL may be altered in DM (Durrington and Sniderman, 2002).

A highly significant correlation between HbA1c and FBS in this study is similar with various previous studies (Khaw *et al*., 2004, Ram *et al*., 2011, Masram *et al*., 2012). Significant correlations were observed between HbA1c and TC, LDL-C and TG. In various studies, HbA1c level was eminent as showing positive correlation with TC, LDL-C and TG in diabetic patients (Khaw *et al*., 2004, Ram *et al*., 2011, Masram *et al*.,

2012). This study also showed a significant correlation between HbA1c and non-HDLC. Non-HDL-C was shown to be the stronger predictor of CVD in diabetic population by 'The Strong Heart Study' (data evaluated by Lu et al), with hazard ratios of 2.23 and 1.80 respectively in male and female (Lu *et al*. 2003).

Moreover, NCEP ATPIII has recommended using Non-HDL cholesterol in assessing CVD risk in patients with diabetes. The correlation of HbA1c with non-HDL-C was found to be more significant than correlation of HbA1c with other lipid parameters except for LDL-C in the study. The measurement of Non-HDL-C is simple which can be conducted even in non-fasting state of patients and can be determined regardless of TG concentration. Hence, Non-HDL cholesterol can be of great value in determining dyslipidemia in diabetic subjects. However, in a prospective cohort study with inclusion of 418 Type 2 diabetic individuals with follow-up until the appearance of a cardiovascular event, Gimeno-Orna JA et al (2005), showed that the main lipid predictor of vascular events was mean TC/HDL-C ratio with hazard ratio (HR) of 1.46. In the same study, the predictive power of the TC/HDL ratio was found to be higher than that of Non-HDL cholesterol and study concluded that TC/HDL-C can be used as a treatment guides for diabetic dyslipidemia.

Total number of apo-B containing particles and small LDL-C Particles are increased in diabetes and these metabolic abnormalities are better reflected by TC/HDL-C ratio and Non-HDL-C than LDL-C alone (Lemieux *et al*., 2001 and Peters, 2008). Significant association of HbA1c with various lipid parameters, Non-HDL-C, LDL-C/ HDL-C

ratio and TC/HDL-C ratio in this study suggests the importance of glycemic control in order to control dyslipidemia.

The Diabetes complications and control trial (DCCT) established HbA1c as the gold standard of glycemic control. The level of HbA1c value ≤7.0% was said to be appropriate for reducing the risk of cardiovascular complications (Rohlfing et al., 2002) In the present study, diabetic patients were divided into 2 groups as per the HbA1c cutoff of 7.0%. The diabetic patients with HbA1c value > 7.0% exhibited a significant increase in TC, LDL-C, TG, LDL-C/HDL-C ratio, Non-HDL-C and Risk ratio without any significant alteration in HDL-C in comparison to patients with HbA1c value ≤7.0%.

Khan *et al* (2007) showed the impact of glycaemic control on various lipid parameters in which the diabetic patients were categorized into 3 groups according to their HbA1c levels: group 1, good glycaemic control (HbA1c<6%); group 2, poor glycaemic control (HbA1c >6%–9%) and group 3, worst glycaemic control (HbA1c>9%). Though there was no significant differences in LDL–C in 3 groups with regard to glycaemic control, alterations in other lipid parameters were statistically significant in three different groups. Severity of dyslipidemia increases in patients with higher HbA1c value. As elevated HbA1c and dyslipidemia are independent risk factors of CVD, diabetic patients with elevated HbA1c and dyslipidemia can be considered as a very high risk group for CVD. Improving glycaemic control can substantially reduce the risk of cardiovascular events in diabetics (Selvin *et al*., 2006)

Significant correlations between HbA1c and the lipid parameters and a linear relationship between HbA1c and dyslipidemia point towards the usefulness of HbA1c for screening high-risk diabetic patients. Furthermore, there were no significant interactions between sex or age and HbA1c with respect to lipid profile suggesting the validity of HbA1c for predicting dyslipidemia irrespective of patient's gender and age.

Chapter 6

CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

Significant correlation between HbA1c and various circulating lipid parameters and significant difference of lipid parameters in two groups (≤7.0% and >7.0%) of glycated hemoglobin indicates that HbA1c can be used as a potential biomarker for predicting dyslipidemia in type 2 diabetic patients in addition to glycemic control hence early diagnosis can be accomplished through relatively inexpensive blood testing and may be utilized for screening high-risk diabetic patients for timely intervention with lipid lowering drugs.

6.2 RECOMMENDATION

The study indicated a positive correlation between HBA1C and mean duration of diabetes. This suggests that most of the complications of diabetes including

cardiovascular diseases appear with increasing duration of disease, and as such it is of outmost importance that the longer a patient has diabetes, the more comprehensive the care of such patients should be. A conscious effort should be made to detect and manage complications.

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