ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF ALLIED HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Insulin Resistance and Dyslipidemia in Type 2Diabetic Patients: A Cross-Sectional Study at the Diabetic Clinic Of Tikur Anbessa Specialized Teaching Hospital

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A Thesis Presented To Graduate Studies Program, Addis Ababa University In Partial Fulfillment Of The Requirements For The Degree Of Masters Of Science in Clinical Laboratory Science

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INSULIN RESISTANCE AND DYSLIPIDIMIA IN TYPE 2 DIABETIC PATIENTS:ACROSS SECTIONAL STUDY AT THE DIABETIC CLINIC OF TIKUR ANBESSA SPECIALIZED TEACHING HOSPITAL

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LIST OF ABBREVIATIONS

ApoA-I	Apo lipoprotein A-I
ApoB	Apo lipoprotein B
BMI	Body Mass Index
CAD	Coronary artery disease
DKA	Diabetic ketoacidosis
DM	Diabetes mellitus
GLUT 4	Glucose transporters 4
HDL-c	High density lipoprotein cholesterol
HOMA-IR	Homeostasis model assessment-insulin ratio
IGT	Impaired glucose tolerance
LDL-c	Low density lipoprotein cholesterol
MODY	Maturity onset diabetes of the young
ROS	Reactive oxygen species
RXR	Retinoic acid receptor protein
TASH	Tikur Anbessa Specialized Hospital
TG	Triacylglycerol/ triglyceride
WHO	World Health Organization
MODY	Mature onset of diabetes in the young
LADA	Latent autoimmune diabetes in adults

Acknowledgements

First and foremost, I would like to express my gratitude to the almighty of God. This study received approval from the Departmental research and Ethics Review Committee (DRERC) of medical laboratory technology department. I would like to express my gratitude to all those who gave me the possibility to complete this thesis.

I would like to express my gratitude towards my advisors, Ato Mister Wolde, Dr. Daniel Seyfu, Dr Frank Ashall and Dr.Tedla Kebede (Internist), for sharing their enormousknowledge and in reviewing the thesis from their thoughtful knowledge helpedme in accomplishing this work.

I am really thankful for the study subjects, for their voluntary participation in thisstudy. I am also indebted to all staff members of the Department of Biochemistry, and especial thanks Ato Samuel kinde,nurses of diabetes clinic of TASH for their support during samplecollection.

I am really adoringly thankful to our Mother Asegedech Dagne and my beloved husband Teshome Worku for being with me through all the ups and downs during my study.

Abstract

Background: *Type 2 diabetes is a major non-communicable disease with an increasing prevalence in Ethiopia and worldwide. About 2% of Ethiopians are reported to have type 2 diabetes, but this may be an underestimate, due to a paucity of studies on diabetes epidemiology in Ethiopia.*

Objective: *Assessment of insulin resistance and dyslipidemia in type 2 diabetic patients attending diabetic clinic of the Tikur Anbessa Hospital, Addis Ababa.*

Methods: *This study was carried out on 106 type 2 diabetic patients attending Tikur Anbessa Specialized Hospital Diabetic Clinic. Fasting blood glucose, serum lipid panels, serum insulin concentrations, Apo lipoprotein A-1 and Apo lipoprotein B were measured in diabetics who were taking oral medications. Patients taking insulin were excluded from the study. Insulin resistance was measured by calculating HOMA-IR for each patient. Blood pressure, body mass index (BMI), waist circumference and smoking status were also determined.*

Result: *Most patients (80%) had poorly controlled fasting blood glucose (>126 mg/dL), and this was similar for males and females. Serum triglycerides were abnormally high in 58.7% of patients, LDL-cholesterol levels were elevated above 100 mg/dL in 50% of patients, and serum HDL-c levels were abnormally low in 76.9 % of patients.88.6% of the diabetic patients had showed significant insulin resistance ($P<0.05$), indicated by an elevated HOMA-IR index (>3.0).*

Two third of the diabetic patients showed normal blood pressure. Furthermore, sixteen of the 106 patients were cigarette smokers and smokers over 40 years old with diabetes had substantial cardiovascular risks as determined by their Framingham cardiovascular risk score.

Conclusion: *These results emphasize the need for improvedprograms for diabetes screening, prevention and care in Ethiopia. The biochemical and medical significance of the results are discussed.*

Key words: *Type2Diabetes,Insulin Resistance, Dyslipidemia, HOMA-IR, Apolipoprotine.*

CHAPTER 1: INTRODUCTION

1.1 Diabetes: Classification, Definition and Recent Concepts

Diabetes mellitus (DM) is characterized by abnormally high levels of glucose in the blood. When the amount of blood glucose increases, after a meal for example, it triggers the release of the hormone insulin from the pancreas. Insulin stimulates muscle and fat cells to take up glucose from the blood, lowering blood glucose to normal level. In diabetics, blood sugar level remains high. This may be either because insulin is not being produced at all, is not made at sufficient levels, or is not as effective in its actions on peripheral tissues as it should be. The term diabetes mellitus describes a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both [1, 2,3].

The two main types of diabetes are considered to be type 1 diabetes, often occurring in children and involving immune destruction of pancreatic beta cells, leading to early-onset insulin-requiring diabetes, and type 2 diabetes, initially involving resistance of peripheral tissues to insulin, with insulin excess, and later progressing to pancreatic beta cell destruction and insulin deficit. Classic type 1 diabetes results from destruction of the beta cells of pancreas, leading to an absolute insulin deficiency. It usually occurs in children and young adults and absolutely requires insulin treatment. Classic type 2 diabetes may range from predominantly insulin resistance with relative insulin deficiency to a dominantly pancreatic beta-cell secretory defect with or without insulin resistance [3].

Type 2 diabetes is the most common form of diabetes; its chance of occurrence increases with age, family history in a first degree relative, and the Metabolic Syndrome (see later) and obesity are strong risk factors for classic type 2 diabetes. Many patients with type 2 diabetes progress to a stage, due to beta-cell dysfunction, at which they need insulin treatment [3].

Though diabetes is often classified as type 1, type 2, other specific types, and gestational diabetes and this classification of diabetes was widely used, data is accumulating to support the concept that diabetes really is a complex group of perhaps hundreds of diseases, all of which have a common feature of pancreatic beta cell dysfunction leading to a deficit of insulin [1]. For example, latent autoimmune diabetes in adults (LADA) is often clinically misdiagnosed as type 2 diabetes, but it resembles classical type 1 diabetes in some of its characteristics (for example,

presence of anti-Islet antibodies and eventual destruction of beta cells leading to insulin-dependence), yet it is a slow-onset insulin-requiting diabetes that gradually happens over time and affects primarily adults over 35 rather than children and teens [4].

“Mitochondrial” diabetes is due to genetic defects in multiple possible mitochondrial functions and in some cases does not involve pancreatic cell death or insulin resistance: its main etiology is poor mitochondrial signaling of the glucose-induced insulin secretion by pancreaticβcells [5]. Similarly, mature onset of diabetes in the young (MODY) is an inherited disease that resembles type 1 or type 2 diabetes, depending on the specific cause. One form of MODY, for example, occurs due to mutations in β-cell glucokinase, and this causes poor signaling of glucose-mediated insulin secretion by the pancreas. MODY usually does not involve beta cell destruction or insulin resistance, as “classical” type 1 and type 2 diabetes do [6].

It has been proposed that type 2 diabetes is a heterogeneous group of perhaps hundreds of separate diseases, based on the findings that there are groups of individuals who lack obesity, metabolic syndrome or insulin resistance yet develop diabetes that responds to conventional oral ant diabetics normally used for type 2 diabetics and not for type 1 diabetics[7]. Likewise, some obese individuals appear to be resistant to developing type 2 diabetes even though they have insulin resistance [8, 9].

It is argued that the common “type 2 diabetes” seen in western and obese individuals involves a genetic predisposition to pancreatic beta-cell dysfunction, and that insulin resistance merely triggers beta-cell dysfunction in these patients, and that without the genetic predisposition the insulin-resistant individuals would not develop type 2 diabetes [1].

As the need for insulin rises in the most common, classical form of type 2 diabetes, the pancreas gradually loses its ability to produce insulin. Type 2 DM includes a defect in insulin-mediated glucose uptake in skeletal muscle, a disruption of secretary function of adiposities, a dysfunction of pancreatic β-cells, impaired sensing and response to hyperglycemia in the CNS, an excessive accumulation of lipids, and impaired fatty acid oxidation due to obesity, physical inactivity, and genetic predisposition [3].

Type 2 diabetes mellitus is a polygenic disorder that involved numerous genes interacting with environmental factors and is associated with older age, obesity, family history of diabetes, prior history of gestational diabetes, impaired glucose tolerance, physical inactivity, the Metabolic Syndrome and race/ethnicity. Diabetes can also be caused by certain medications, including corticosteroids and atypical antipsychotics [3].

Gestational diabetes mellitus (GDM) is a form of glucose intolerance that is diagnosed in about 7% of women during pregnancy, and is probably related to changes in hormones during pregnancy. It is also more common among obese women and women with a family history of diabetes. Most GDM patients do not develop DM later in life, but some will develop impaired glucose tolerance, type 2 DM, or even type 1 DM. After pregnancy, 5% to 10% of women with gestational diabetes are found to have type 2 diabetes. Women who have had gestational diabetes have a 20% to 50% chance of developing diabetes in the next 5-10 years. Because increased fetal mortality and morbidity have been associated with GDM, prompt detection and aggressive treatment during pregnancy are important [10].

Because the onset of type 2 diabetes is slow and gradual, and most patients with type 2 DM are asymptomatic, type 2 DM can remain undiagnosed for years, and may even present with complications. Visceral obesity (fat around abdominal organs such as liver and intestines) is at least partly responsible for type 2 diabetes, mediated by adipokines produced by visceral fat, causing inflammation and insulin resistance. The β-cells compensate for this resistance by increasing insulin secretion and maintaining normal glucose tolerance. Eventually, the hyperglycemia worsens, glucose toxicity ensues, and insulin secretion and action decrease. Ultimately, the loss of β-cell mass can lead to insulin dependency [10].

1.2 Medical Complications of Diabetes

Diabetes is associated with serious complications, which can be acute or chronic. Acute complications include diabetic ketoacidosis (DKA), which is a medical emergency and caused by excess ketone body production as a result of uncontrolled diabetes [11]. The major chronic complications of diabetes are the micro vascular andmacrovascular complications. Micro vascular complications include diabetic retinopathy, diabetic nephropathy and diabetic neuropathy. Macrovascular complications include coronary artery disease, leading to heart attacks), cerebrovascular disease (leading to strokes), and peripheral vascular disease [11].

Several prospective studies based on a large number of type 2 diabetic patients have established that proper glycemic control is important for preventing diabetic micro vascular complications (retinopathy, nephropathy and neuropathy) and, to some degree, cardiovascular risk. Two landmark studies that have given useful information in defining the risk of insulin resistance for micro vascular and macrovascular complications of diabetes (including coronary heart disease) are the U.K. Prospective Diabetes Study, UKPDS Another study also found significant reductions in micro vascular (retinopathy, nephropathy and neuropathy) complications of diabetes, when blood glucose was well controlled in diabetics[12].These studies showed a large reduction (35% to 75%) in the risk of micro vascular complications in diabetic patients associated with normalization of plasma glucose. The UKPDS, based on 2,693 patients with newly detected type 2 diabetes, aged 30–50 years, also evaluated the significance of all major cardiovascular risk factors for CHD. The most important risk factor for CHD was high LDL cholesterol, followed by low HDL cholesterol [12].

Subjects with type 2 diabetes have higher cardiovascular risk than non-diabetics. Most studies have indicated that this excess risk for macro vascular complications cannot be explained by abnormal levels of conventional cardiovascular risk factors, and the diabetes state itself, particularly hyperglycemia, is likely to contribute to excessive cardiovascular risk in patients with type 2 diabetes. Prevention of macro vascular complications of diabetes does not appear to be as good with glycemic control alone of diabetes as micro vascular complications, but other cardiovascular risk factors in diabetics, such as hypertension, dyslipidemia and smoking cessation, must be aggressively managed in diabetics [12].

A recent report Showed that rates of all of five diabetic complications studied (lower-extremity amputation, end-stage renal disease, acute myocardial infarction, stroke, and death from hyperglycemic crisis) declined in the USA between 1990 and 2010. In particular, there was a 67% drop in the rate of myocardial infarction. These declines are likely due to improved preventive medical practices, such as screening for diabetes and patient education, even though the prevalence of diabetes continues to increasethis study stresses the importance of diabetes screening in the general population and aggressive education of patients and healthcare workers in management of diabetes [13].

1.3 Non-communicable Diseases (NCDs) in Ethiopia

Non-communicable diseases (NCDs) comprise four major groups of chronic diseases: cardiovascular diseases, cancers, chronic lung diseases and diabetes[14].About 80% of NCDs are caused by four major behavioral risk factors: cigarette smoking, alcohol use, lack of exercise and poor diet [14].

Though they are often distinguished from communicable diseases, the dividing line between the two is not clear cut. Thus, some NCDs can be caused by infectious agents and are therefore really communicable, for example liver cancer can be caused by hepatitis B and C viruses, and cervical cancer is usually caused by human papilloma virus. In addition, there are strong interactions between NCDs and classical communicable diseases; for example, smokers are more likely to contract HIV and tuberculosis, and tuberculosis patients who smoke are more likely to develop medical complications and die from their tuberculosis [14].

It has been suggested that NCDs and communicable diseases should be treated together as co-epidemics rather be dealt with separately. This may be particularly true of Ethiopia and other low- and middle-income countries, where NCDs are becoming an increasing burden, as communicable diseases, malnutrition and maternal-childbirth health issues have dominated healthcare focus [14, 15].

Indeed, 80% of deaths due to NCDs occur in low- and middle-income countries and 34% of Ethiopians will die from an NCD [16].Ethiopia has a high prevalence of NCDs and these are increasing. An analysis of 32 published studies, a half of them done in Addis Ababa, found varying prevalence of NCDs depending on the location and study, but all NCDs were present at significant levels. Cardiovascular disease (prevalence from two studies, 7.2% and 24%); cancer (prevalence 0.3%); diabetes (prevalence 0.5% and 1.2% from two studies); asthma (prevalence 1 to 3.5 %). About a quarter of deaths in Addis Ababa were due to cardiovascular disease, and 10% of deaths in urban areas of Ethiopia were due to cancer [16].

Smoking is said to be low (7% in men and 0.9% in women) in Ethiopia, but in some areas it is high; for example, in one rural area of eastern Ethiopia, 28 % of adults studied reported being a smoker [17].Tobacco use is an independent risk factor for cardiovascular diseases and is particular risky in diabetics, so more data is needed on smoking prevalence in the general population as well as specifically in diabetics in Ethiopia [17].

Hypertension and dyslipidemia are also common treatable risk factors for NCDs, especially for cardiovascular disease. Diabetics with hypertension and/ or dyslipidemia need to be aggressively managed in order to reduce their cardiovascular outcomes. Again, dyslipidemia and hypertension are common in Ethiopia, but studies show variability from one study to another found a prevalence of hypertension of 22% in Ethiopian adult men and 14.9% in women. Dyslipidemia and other characteristics of Metabolic Syndrome are also common in Ethiopia [18, 19].Risk factors for cardiovascular diseases are not only common in the Ethiopian population, but also often individuals have multiple risk factors. In a study of 950 diabetic patients in Jimma University Socialized Hospital, hypertension was present in 46.5%, obesity in 23.4%, dyslipidemia in 63.5%, physical inactivity in 55% and cigarettesmoking in 5.5% of the diabetics [20]. In another study of 305 diabetics in Jimma, 96.1% of patients with type 2 diabetes mellitus had hypertension [21].

1.4 Epidemiology of Diabetes

Over the last 30 years, type 2 diabetes has changed from a relatively mild disease associated with aging to one of the most prevalent causes of premature mortality and morbidity in most countries. The global increase in type 2 diabetes is largely due to increased prevalence of overweight and obesity, as a result of poor diet and exercise practices, and has increased in teenagers in the USA and other countries where teenage obesity has become more prevalent. Estimates from the 2009 International Diabetes Federation (IDF) suggest that the number of adults living with diabetes in the world will expand by 54%, from 284.6 million (6.4%) in 2010 to 438.4 million by 2030. The projected growth for sub-Saharan Africa (SSA) is that diabetes prevalence will double from 12.1 million in 2010 to 23.9 million in 2030. Other predictions are that there may be as many as 550 million cases of diabetes globally by 2015 [22, 23, 24, and 25].

The prevalence of diabetes in other sub-Saharan African countries varies widely and data is again sparse [24].One study found the following diabetes prevalence: urban Sudan (3.9%), urban South Africa (6 to 8%), Ghana (6.4%), and Kenya (4.2%). Diabetes was estimated to cause 6% of all deaths in sub-Saharan African in 2010, but this is likely to increase with the increasing prevalence of diabetes. Most (90%) diabetes in Sub-Saharan Africa is type 2, though whether this diabetes is identical to the predominant type 2 diabetes seen in higher-income, more obese, countries remains to be determined [24]. Another survey of sub-Saharan Africa showed a diabetes prevalence ranging from 1% in rural Uganda to 12% in urban Kenya [25]. Type 1 diabetes prevalence was low and ranged from 4 per 100,000 in Mozambique to 12 per 100,000 in Zambia. Gestational diabetes prevalence varied from 0% in Tanzania to 9% in Ethiopia. This high prevalence of gestational diabetes in Ethiopia needs to be confirmed and, if true, needs to be addressed because of its link with neonatal morbidity and mortality. Proportions of patients with diabetic complications ranged widely: retinopathy (7 to 63%), neuropathy (27 to 66%), and micro albu8iminuria (10-83%). Poor access to diagnostic tools and glucose monitoring equipment and high cost of diabetes treatment are general barriers to dealing with diabetes in sub-Saharan Africa [25].

Data on diabetes prevalence in Ethiopia is relatively sparse the estimated prevalence of DM in adult population of Ethiopia is 1.9% [20, 21]. Estimated the prevalence from two different hospitals to be 0.5% and 1.2%, respectively. However, reported a high prevalence of diabetes (8.9%) among Ethiopian Jews less than 30 years old that migrated to Israel. However, these data may significantly underestimate the prevalence of type 2 diabetes in Ethiopia, because screening of diabetes in the general population is rarely done in Ethiopia, and most type 2 diabetic patients are asymptomatic for many years before they show symptomatic complications [22]. The reality is, therefore, that the precise incidence and prevalence of diabetes in Ethiopia is unknown. A cross-sectional study of over 2,000 Ethiopian adults revealed a prevalence of diabetes of 6.5 % for men and 6.4 % for women and this may be a more accurate estimate, because it was done a larger sample number [23].

Diabetic complications are also significantly prevalent in Ethiopian diabetics. Diabetic ketoacidosis was common in type 1 diabetics in a Jimma hospital, and almost a half of diabetic patients had proteinuria, 29.5% had peripheral neuropathy, and diabetic foot ulcers (4.5 %), dental problems (10%) and tuberculosis (5.6%) were not uncommon [26].

The Ethiopian Public Health Training Initiative have published a training course for healthcare workers, including health extension workers, summarizing the main issue related to diabetes diagnosis, treatment and management in Ethiopia [27]. Training of skilled healthcare workers and education of the public about prevention and treatment of diabetes will crucial for controlling the diabetes epidemic in Ethiopia [27].

Diabetes is a leading cause of blindness, end stage renal disease and stroke that are two to five times more common among diabetic patients. Diabetes is a growing problem in the developing world including Africa. The International Diabetes Federation (IDF) predicts that most new cases of diabetes will occur in developing nations. According to WHO the rise in cases over the next few decades will approach 200% in developing, and 45% in developed countries respectively. The prevalence of diabetes in traditional rural African communities is less than 1% but escalates up to 30% in cities [28].

No population-based prevalence study exists in Ethiopia but from hospital based studies it can be seen that the prevalence of diabetes admission has increased from 1.9% in 1970 to 9.5% in 1999 of all medical admissions. WHO estimated the number of diabetics in Ethiopia to be about 800,000 cases by the year 2000, and the number is expected to increase to 1.8 million by 2030.Achieving and maintaining normoglycemic, blood pressure and lipids levels in both Type 1 and Type 2 diabetes may require close ongoing support from a health care team, financial resources, and advanced patient knowledge and motivation [28].

Type 2 diabetes over the next two decades, the largest increase in the number of people with diabetes will be seen in developing countries, particularly in people of working age. Complications of T2DM are mainly associated with diabetic vasculopathy, which are commonly grouped into two categories, viz., micro vascular (retinopathy, neuropathy and nephropathy) and macrovascular (which puts the diabetic patients at increased risk of cardiovascular disease). The overall temporal burden of hyperglycemia is responsible for diabetic complications and adverse outcomes. Although, with the advances in medical practice and technology, the overall risk of mortality from the cardiovascular disease has decreased, the diabetes mellitus patients continue to display distressingly high morbidity and mortality due to coronary events. The increased vascular risk associated with T2DM is likely to be multifactorial, but dyslipidemia, now called as _diabetes lipids, plays an important role. It is important to note that dyslipidemia in diabetic patients is more atherogenic than that in non-diabetics [29].

1.5 The Metabolic Syndrome, Obesity and Visceral Fat

Metabolic syndrome includes the constellation of various metabolic abnormalities and confers an increased risk for diabetes and cardiovascular diseases [27]. Studies exploring the prevalence of metabolic syndrome have come up with varied findings, primarily due to differences in cut off points for various components of the syndrome. It is estimated that around 20–25% of the world's adult population has metabolic syndrome. From a physiopathological perspective, this syndrome is associated with multiple metabolic changes, physical findings and hemodynamic disorders, such as reduced glucose tolerance, type 2 diabetes, insulin resistance, arterial hypertension, fatty liver, altered lipid metabolism (hypertriglyceridemia, hypercholesterolemia, reduced HDL-c, and elevated low-den lipoprotein), obesity, waist circumference and abnormalities of the coagulation system[30,31]

A commonly used set of parameters for Metabolic Syndrome includes the ATPIII criteria in accordance with the ATP III criteria, patients are classified as having Metabolic Syndrome if they have three or more of the following risk factors: (1) abdominal obesity (waist circumference >102 cm in males and >88 cm in females); (2) hypertriglyceridemia (TG ≥150mg/dL); (3) reduced HDL-C (<40 mg/dL in males and <50mg/dL in females); (4) high BP (≥130/85mmHg); (5) FBG (≥110mg/dL). Metabolic syndrome includes the constellation of various metabolic abnormalities and confers an increased risk for diabetes and cardiovascular diseases. Studies exploring the prevalence of metabolic syndrome have come up with varied findings, primarily due to differences in cut off points for various components of the syndrome. It is estimated that around 20–25% of the world's adult population has metabolic syndrome [32].

Metabolic Syndrome is also known as insulin resistance syndrome. This syndrome is a cluster of disorders associated with insulin resistance, impaired glucose intolerance and hyperinsulinemic. Insulin resistance appears to be the primary mediator of metabolic syndrome. Insulin promotes glucose uptake in muscle, fat, and liver cells and can influence lipolysis and the production of glucose by hepatocytes. The linked concepts of metabolic syndrome/insulin resistance syndrome have served a highly useful purpose by providing a simple construct to characterize many types of patients who clinicians see daily, and to help identify people at risk. Obesity in particular abdominal adiposity is one of the main reasons for Insulin resistance [31, 32].

Non-esterified fatty acids (NEFA) are released from excess adipose tissues, which increase insulin resistance. In case of insulin resistance there is increased lipolysis from the adipose tissue which increases the free fatty acids, further inhibiting the anti-lipolysis effect of Insulin. Visceral or mental fat appears to be the most detrimental and contributes most to the development of lip toxicity in peripheral tissues by the secretion of adipocytokine [33].

Metabolic Syndrome is associated with a high amount of intra-abdominal fat, low adiponectin levels, and elevated levels of cytokines. Hyperinsulinemic may increase the production of very low-density lipoprotein triglycerides and thus raise triglycerides. Insulin resistance can raise blood pressure. Additional contributors to insulin resistance include abnormalities in insulin secretion and insulin receptor signaling, impaired glucose disposal, and proinflammatory cytokines [34].

Insulin resistance is especially related to visceral fat (fat around organs inside the body, especially abdomen: intestines, kidneys), and less related to fat under the skin (subcutaneous fat). Visceral fat is much more associated with insulin resistance, diabetes and heart disease, than subcutaneous fat. Visceral fat may be a very different "tissue" compared with subcutaneous fat, and produces much higher amounts of Adipokines than subcutaneous fat [33, 34].

Adipose tissue, in particular visceral fat, is itself an endocrine tissue and secretes Adipokines, which are hormones or cytokines that affect other tissues in multiple ways and are responsible at least partly for the development of insulin resistance and cardiovascular disease associated with the Metabolic Syndrome and obesity. There are over 80 Adipokines include leptin, adiponectin, TNF-alpha, IL-6, which regulate appetite, weight, insulin sensitivity, energy expenditure and contribute to insulin resistance in Type 2 diabetes through their effects on peripheral tissue cells. Subcutaneous fat produces lower levels of Adipokines and does not appear to be a higher risk for insulin resistance and diabetes [35, 36].

Insulin resistance refers to the lowered ability of cells to respond to insulin. Peripheral insulin resistance is particularly used to refer to the reduced capacity of muscle and adipose cells in particular to respond to insulin. Insulin resistance frequently accompanies the Metabolic Syndrome and is a component of classical type 2 diabetes. The defect lies in the signaling pathways that are stimulated by insulin binding to its receptor on muscle and adipose cells. Normally insulin stimulates, through signaling pathways, up-regulation of GLUT4 receptors on target cells, so increasing glucose uptake by these cells, but in insulin resistance this pathway is impaired [37.38]. Insulin resistance is a complex metabolic disorder that defies explanation by a single etiological pathway. Accumulation of ectopic lipid metabolites, activation of the unfolded protein response (UPR) pathway, and innate immune pathways have implicated in the pathogenesis of insulin resistance and are associated with fatty acid uptake, lip genesis, and energy expenditure leading to lip toxicity and other abnormalities. They promote the accumulation of toxic levels of diacylglycerols and/or ceramides in liver and skeletal muscle, a common final pathway leading to impaired insulin signaling and insulin resistance [39, 40].

1.6 Dyslipidemia

The term dyslipidemia is used to describe a group of conditions in which there are abnormal levels of lipids and lipoproteins in the blood and is a major risk factor for coronary artery disease (CAD). Patients with type 2 diabetes have a marked increase in the risk of premature CAD. It is known that the same factors play a role in no diabetic individuals also; however, the overall effect of a given risk factor, in terms of increasing risk for cardiovascular disease, is greater in diabetic population [41].

Lipoproteins are complex aggregates of lipids and proteins that render the lipids compatible with the aqueous environment of body fluids and enable their transport in the blood. The most abundant lipid constituents of lipoproteins are triacylglycerol (triglyceride), free cholesterol, cholesterol esters and phospholipids (phosphatidylcholine and sphingomyelin especially), though fat-soluble vitamins and anti-oxidants are also transported in lipoproteins [41].

The interior, hydrophobic part of lipoproteins contains cholesterol esters and triglycerides, whereas free cholesterol and phospholipids occur on the surface of the lipoprotein particle. Proteins, called Apo lipoproteins, are usually found on the surface of lipoproteins and may have enzyme functions or serve as ligands that allow the lipoproteins to bind to specific receptors on cell surfacesin mammals, the lipoprotein transport system serves many functions that are crucial for survival including the initial transport of dietary fats from the intestine to the liver, the secondary transport of processed cholesterol particles to peripheral tissues for steroid hormone production and membrane synthesis, and the processing of free fatty acids which ultimately serve as a source of fuel for immediate and future needs Lipoproteins are classified according to their size and density, with chylomicrons, chylomicron remnants, and VLDL being relatively large and light, whereas LDL and HDL are sequentially smaller and heavier, though there are numerous subpopulations of each type of lipoprotein[42].

In humans, LDL particles are the main carrier of cholesterol to peripheral tissues where they are internalized through the LDL receptor by receptor-mediated endocytosis. Genetic defects that result in loss of function of LDL receptor cause inherited hyperlipidemias. For LDL cholesterol, the associated Apo lipoprotein molecule is Apo lipoprotein B. Figure 1 schematically shows the structure of lipoproteins and their relation to size (diameter) and density [42].

Increased concentrations of serum LDL cholesterol are associated with an increased risk of myocardial infarction and stroke, and reaction of LDL-c with reactive oxygen species is an early step in atherosclerotic plaque formation. Individuals who inherit one copy of a defective LDL receptor-related gene (heterozygous Familial Hypercholesterolemia), if left untreated, often have myocardial infarctions in their 30s and 40s. If a person is homozygous for these mutations (homozygous Familial Hypercholesterolemia), they have extremely high serum LDL-c levels and can have myocardial infarctions in their late teens and early 20s [42].

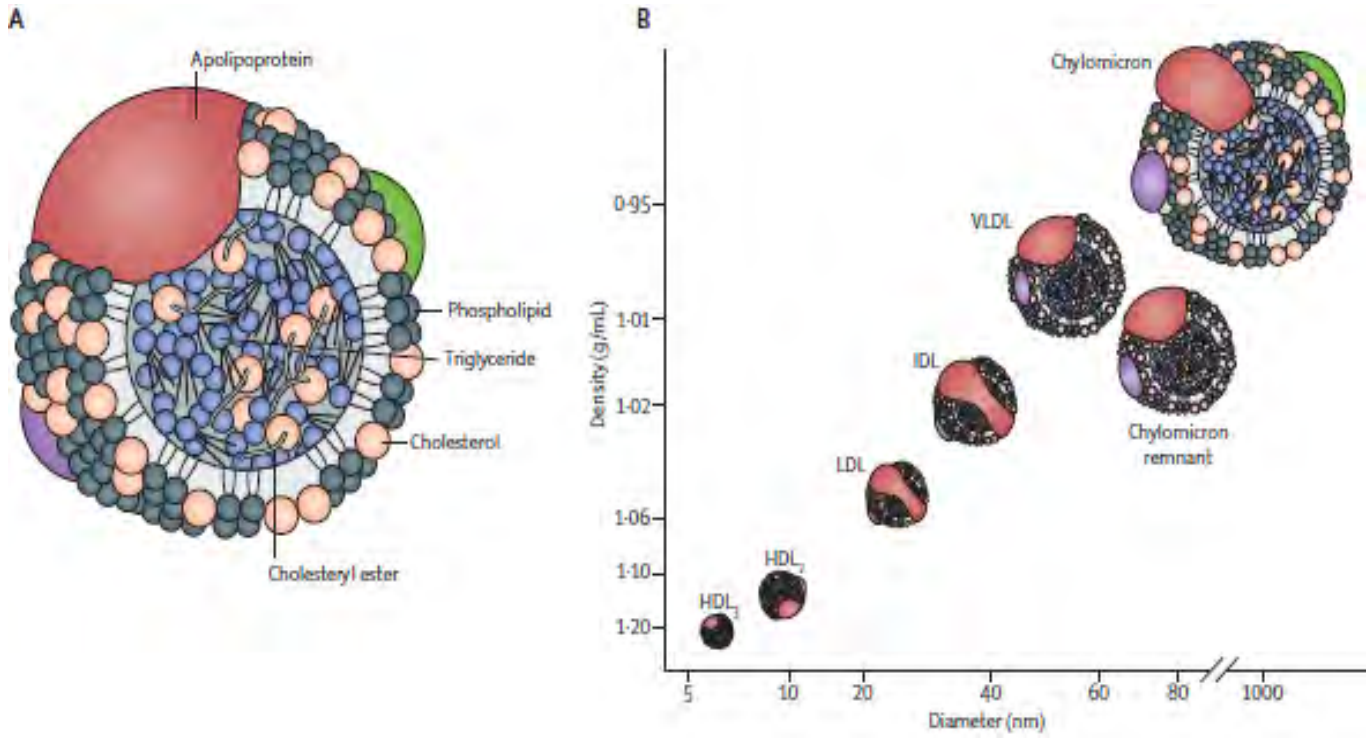

Figure 1.1Structure, types and densities of blood from Braun Wald's Heart Disease: A Textbook of Cardiovascular Medicine, 10th Edition (20150).

High density lipoprotein (HDL-c) contains Apo lipoprotein A-1. Formation of HDL occurs in the liver and intestine, which both synthesize and secrete ApoA-I. Shortly after secretion as a lipid-poor protein, ApoA-I interacts with the cholesterol–phospholipid transporter ABCA1 (ATP Binding Cassette A1) expressed by hepatocytes and enterocytes to acquire lipids, thereby generating a nascent HDL particle [43].

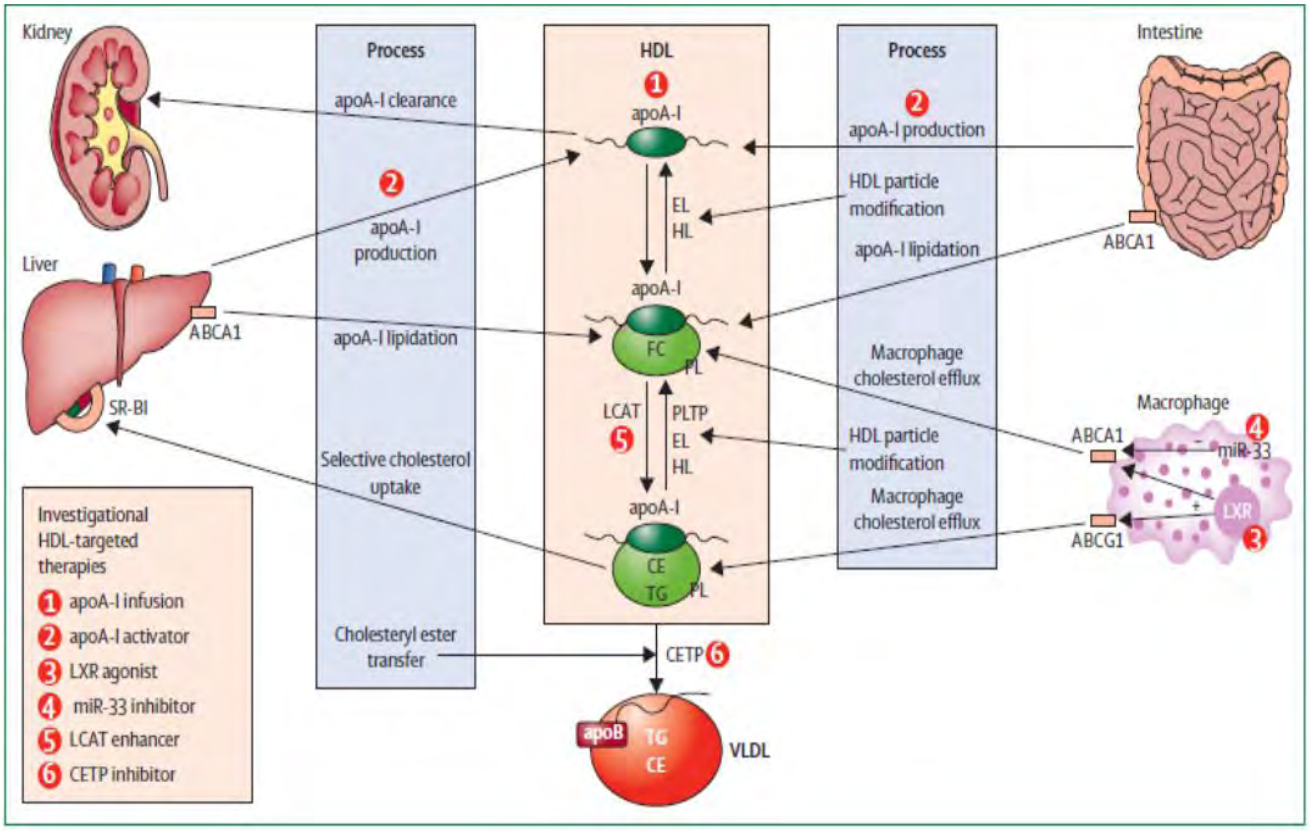

Figure 1.2: HDL-cholesterol metabolism, from Braun Wald's Heart Disease: A Textbook of Cardiovascular Medicine, 10th Edition (2015)

Low serum HDL levels are a risk factor for cardiovascular disease, and elevated HDL are protective against cardiovascular disease. However, pharmaceuticals specifically developed to raise HDL have been disappointing so far in that they have not shown that simply raising HDL is beneficial, and may even be harmful. There are subpopulations of HDL particles and some of these are harmful whereas others are beneficial, and research is ongoing to try and understand HDL subpopulations and metabolism in order to understand the complexities of HDL [44].HDL acquires additional lipids and Apoipoproteins derived from the hydrolysis of triglyceride-rich lipoproteins, and this process partly accounts for the strong inverse relation between triglycerides and HDL-C [44].

1.7Apo lipoprotein A-I and B

Apo lipoprotein A-I and Apo lipoprotein B are the major Apoipoproteins of high-density lipoprotein (HDL) and low-density lipoprotein (LDL), respectively. ApoB, a measure of non-HDL cholesterol, is involved in forming VLDL in the liver and ApoB100, which is the Apoipoproteins of LDL particles, mediates the attachment of LDL to arterial walls, whereas ApoA is involved in biogenesis and function of HDL, which is a ―scavenger" of cholesterol from peripheral tissues. Elevations of ApoA-1 (a measure of HDL levels) have been reported to be inversely associated with cardiovascular disease risk, and elevated ApoB have been implicated in coronary artery disease risk. The ratio ApoB: ApoA-1 appears to be an even better predictor of CAD than either lipoprotein alone. Nevertheless, these are still not widely used in clinical practice .In this study, serum apoA-1 and ApoB assay kits were used to assess diabetics in TikuAnbessa Hospital [45, 46]

Table 1.1. Major Categories of Apoipoproteins

Apoipoproteins	Lipoproteins	Function(s)
Apo B-100	VLDL, IDL, LDL	Secretion of VLDL from liver Structural protein of VLDL, IDL, and HDL Ligand for LDL receptor (LDLR)
Apo B-48	Chylomicrons, remnants	Secretion of chylomicrons from intestine; lacks LDLR binding domain of Apo B-100
Apo A-I	HDL, chylomicrons	Major structural protein of HDL Activator of LCAT

1.8 Significance of the Study

Insulin resistance and dyslipidemia are two common features seen in most type II diabetes mellitus patients. These features, if not diagnosed at the early stages, may result serious medical complications. Few studies have been done in Ethiopia on dyslipidemias and insulin resistance in diabetics. This study, therefore may be of great value for clinicians, healthcare workers and researchers who want to know more about diabetic control, lipid profiles, blood pressure control, BMI and waist circumference data, and insulin resistance in known type 2 diabetic patients in Ethiopia.

CHAPTER 2: OBJECTIVE

2.1 General Objectives

Assessment of insulin resistance and dyslipidemia in type 2 diabetic patients attending diabetic clinic of the Tikur Anbessa Hospital, Addis Ababa.

2.2. Specific objectives

- To assess and compare insulin resistance, using HOMA-IR, in type 2 diabetic patients.
- To assess glycemic control, lipid abnormalities, Apo lipoprotein A-1 and Apo lipoprotein B, in type 2 diabetic patients.
- To compare insulin resistance and other biochemical parameters between diabetics with good and poor glycemic control.
- To examine the status of blood pressure, smoking status, waist circumference and BMI in the diabetic patients.

CHAPTER3: MATERIALS AND METHODS

3.1 Study Area

- Diabetes Clinic of Tikur Anbessa Specialized Hospital (TASH), Addis Ababa.

3.2 Study population

- Diabetic outpatients attending the Diabetes Clinic of Tikur Anbessa Specialized Hospital (TASH), Addis Ababa

3.3 Study subject

The study was designed to include type 2 diabetic patients (male and female) who were willing to give informed consent and full fill inclusion criteria.

3.4. Study period

Sample collection periodwas from September 2015- November 2015.

3.4. Inclusion and exclusion criteria

Inclusion criteria

- Type 2 DM patients visiting TASH diabetic clinic, school of medicine, college of health sciences

Exclusion criteria

- Type 1 diabetic and any diabetic taking insulin as a medication
- Age range beyond 55
- Pregnant or lactating Women.
- Subjects who had other debilitating chronic disease or were on chronic corticosteroids, which could interfere with the blood analysis and interpretation of the data.

A questionnaire (Annex I) was filled after obtaining informed consent from the type 2 diabetic subjects. The information from the questionnaire was recorded and used for the study.

3.5 Sample Size Determination

The sample size is calculated based on the International Diabetic Federation's (IDF) stated 3.5% prevalence of type 2 diabetes in Ethiopia. The calculated sample size using the following formula is 106 type 2 diabetes patients.

$$N = \frac{(Z\alpha/2)^2 \; P(1-P)}{d^2}$$

$$N = (1.96/0.035)2\ 0.035(1-0.035) = 106$$

Where N- number of patients, Z- confidence interval (95%), P – Proportions 3.5%, d- the level of confidence is 5%.

3.6 Serum Sample Collection

For serum preparation, 10 mL of venous blood were collected from antecubital veins of patients after an overnight fast and before morning oral glycemic medications were taken. The blood was collected in sterile serum-separating tubes without anticoagulant and left to form a blood clot at room temperature for 30 minute, then centrifuged with 3500 revolution per minute for 10 minutes. The serum was transferred to sterile tubes and stored in a deep freezer at minus 70°C until analysis.

3.7 Ethical Considerations

This study received approval from the Departmental Research and Ethics Review Committee protocol number: DRERC058/13/MLS of medical laboratory technology department. An information sheet was given to all subjects who agreed to participate. Informed consent was obtained from all subjects on a prescribed form. The confidentiality of the study data was maintained by coding of samples and data.

3.8 Body Mass Index, Waist Circumference

Patient‘s weight was measured using a standard balance, and height was measured using a height measuring device attached to the balance. Body Mass Index (BMI) was calculated from the body weight (kg) and height (meter) as follows:

$$BMI = Weight\ (in\ kg) / (Height\ in\ m)^2$$

Based on the National Institutes of Health Guidelines on Overweight and Obesity (2014), subjects with BMI below 18.5 kg/m2 are classified as ―underweight," BMIs from 18.5 kg/m2 to 24.9 kg/m2 as classified as ―normal," BMIs from 25.0 to 29.9 as classified as ―overweight," and BMIs at or above 30.0 kg/cm2 are considered ―obese."

Waist circumference was measured at the superior level of the umbilicus using a flexible tape measure. This corresponds with the midway point between the iliac crest and the lowest point of the lowest rib. A high waist circumference (over 35 inches in women and over 40 inches in men) is associated with an increased risk for type 2 diabetes, dyslipidemia, hypertension, and CAD in patients with a BMI in a range between 25 and 34.9 kg/m2 [47]. Waist-to-hip ratio has also been associated with these abnormalities, but data suggests that measuring hip circumference adds very little further value to waist circumference alone [48].

3.9 Determination of Fasting Blood Glucose Level

Glucose levels in the patients‘ serum samples were estimated by using a commercial kit based on the method developed by Coxon and Schaffer obtained from Fluitest® GLU, Germany [49].

3.9.1 Principle: Oxidation of glucose by glucose oxidase generates D-gluconolactone plus hydrogen peroxide. The hydrogen peroxide generated then reacts with phenol and 4-aminoantipyrine in the presence of the enzyme, peroxidase, to form a colored quinoid dye product. The absorbance of this colored product is proportional to the glucose concentration in the original sample, and was measured at 546 nm.

3.9.2 Reagents: R1: 150 mmol/L Phosphate buffer pH 7.5, containing 7.5 mmol/l phenol , 12,000 U/l Glucose Oxidase, 660 U/L Peroxidase, 0.40 mmol/l of 4-aminoantipyrine R2: 100 mg/dl (5.55 mmol/l) of glucose standard solution.

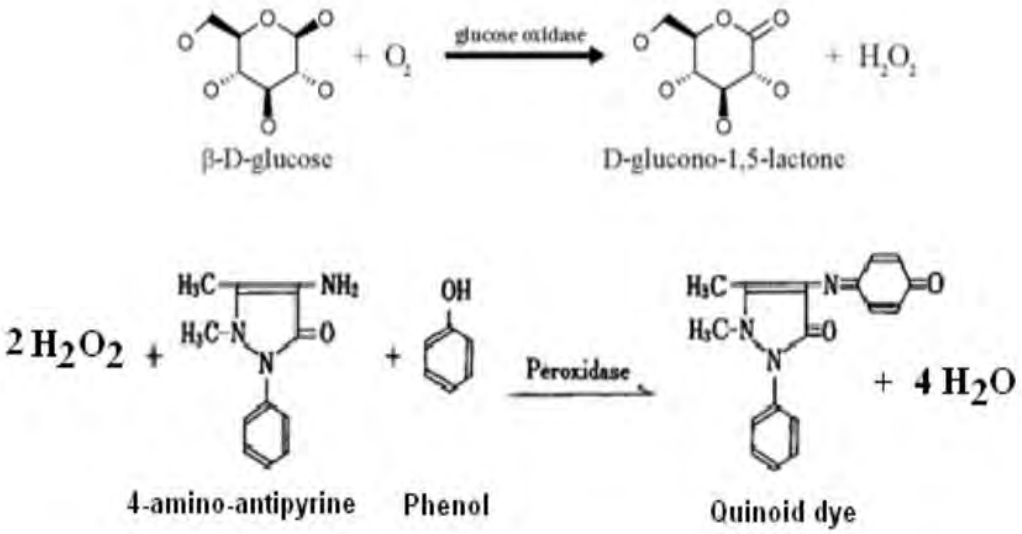

Figure 3.9Principle of the glucose assay

3.9.3 *Procedure:* To labeled test tubes, 1.0 ml of reagent R1 was mixed with 10 µl of sample or glucose standard (R2). After 15 minute of incubation at 370C, the absorbance of sample and standard were measured at 546 nm against reagent blank by using a 5010 spectrophotometer [50].

3.9.4 Calculation of Glucose concentration

Glucose (mg / dL) = (A sample / A standard) X concentration of the standard

Where A is Absorbance at 546nm.

3.10Determination of Serum Total Cholesterol Level

Serum total cholesterol was estimated by using a commercial kit, based on the method developed by Coxon and Schaffer [51].

3.10.1 *Principle:* The method for the measurement of serum total cholesterol involves the use of three enzymes: cholesterol esterase (CE), cholesterol oxidase (CHOD) and peroxidase (POD). Cholesterol esters are first hydrolyzed to release free cholesterol and triglycerides using cholesterol esterase. The free cholesterol is then oxidized by CHOD to generate H2O2. The hydrogen peroxide reacts with phenol and 4-aminoantipyrine in the presence of peroxidase to generate a colored quinoid dye product, the absorbance of which is measured at 546 nm, and is proportional to the concentration of total cholesterol in the original sample (Figure 4).

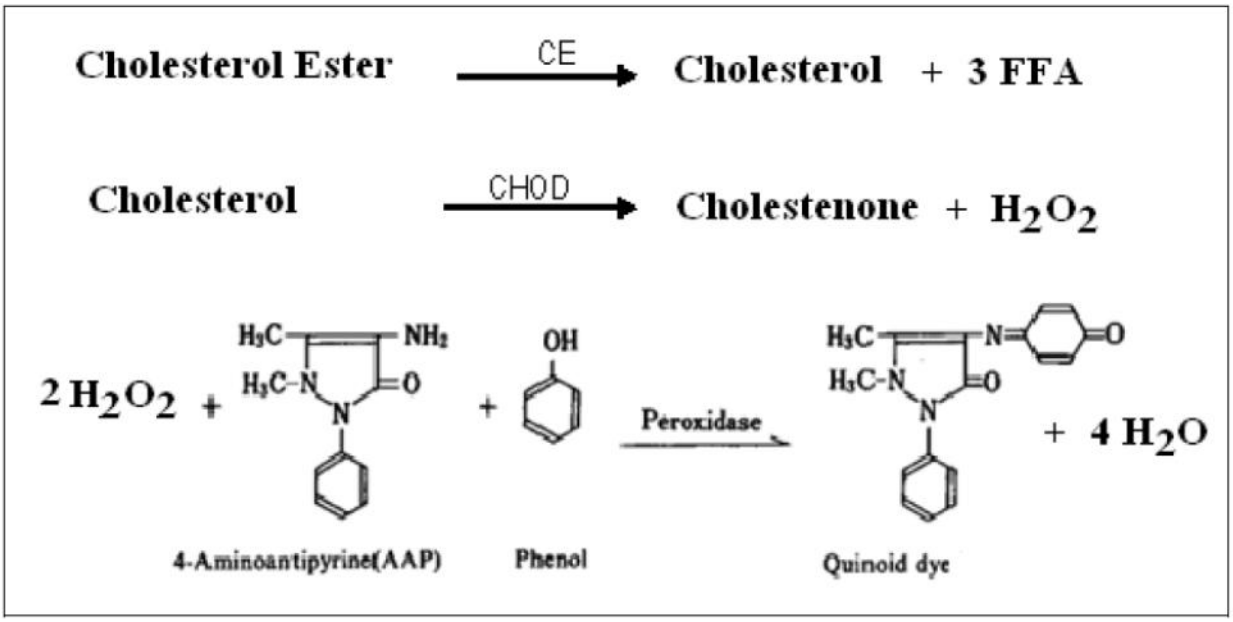

Figure 3.10 Principle of assay for determination of serum total cholesterol

3.10.2 Reagents: R1: 200 mmol/L PIPES pH 7.0, containing 1 mmol/L sodium cholate, > 250 U/L cholesterol esterase, >250 U/L cholesterol oxidase, > 1 KU/L peroxidase, 0.33 mmol/L 4-aminoantipyrine, 4 mmol/L phenol, 2 g/L non-ionic surfactant, and commercial biocides.
R2: 5.18 mmol/L cholesterol standard

3.10.3 Procedure: To the labeled test tubes, 1.0 ml of the working reagent (R1) was mixed with 10 µl of serum sample or 10 µlof standard cholesterol solution. After 5 minutes of incubation at 370C, the absorbance was measured at 546 nm against the reagent blank.

3.10.4 Serum Total Cholesterol Concentration Calculation: Serum total cholesterol concentration is calculated as follows:

Total Cholesterol (mg/dL) = (A sample / A standard) X Concentration of standard

Where A is the absorbance at 546 nm of solutions after reactions were completed.

Table 3.1. Risk stratification of total serum cholesterol levels

Total Cholesterol	Risk Classification
<200	**Desirable**
200-239	**Borderline/high**
>240	**High**

3.11 Determination of Serum Triglycerides

Serum triglycerides were estimated by using a commercial kit, based on the method developed by obtained from Cromatest® Cholesterol MR, Linear chemicals SL, Barcelona, Spain [52].

3.11.1 Principle: The method is based on the initial enzymatic hydrolysis of triglycerides to glycerol and free fatty acids (FFA) by lipoprotein lipase (LPL), followed by three further enzyme steps that eventually produce a colored product that is quantitatively proportional to the concentration of triglyceride in the serum sample being tested. The glycerol generated is phosphorylated by glycerol kinase (GK) to glycerol-3-phosphate, which is then oxidized by glycerol phosphate oxidase (GPO) to form dihydroxyacetone phosphate (DHAP) and hydrogen peroxide (H2O2). With peroxidase (POD) and H2O2, the chromogenic substrate,
4-aminoantipyrine, is coupled with phenol to form a colored product, whose optical density is measured at 500 nm.

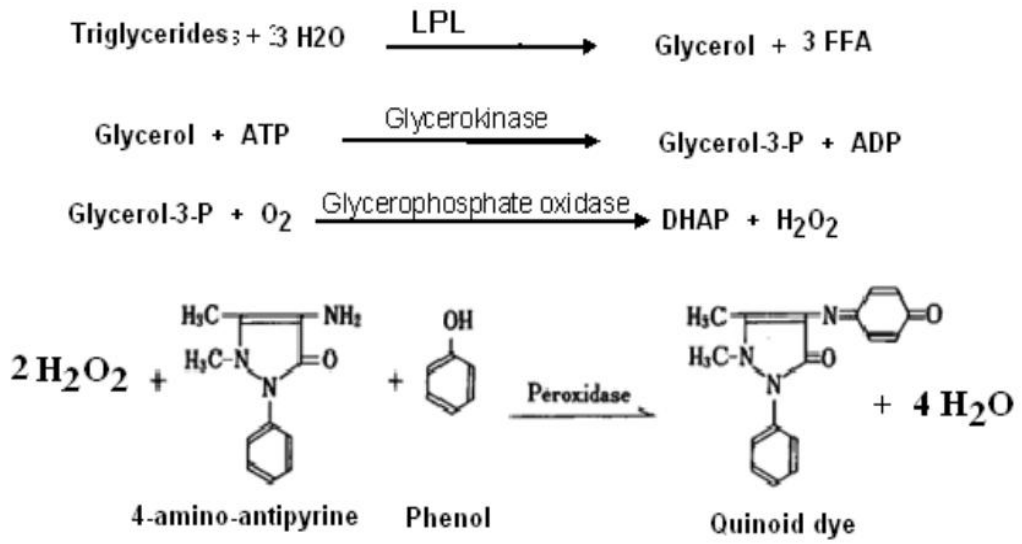

Figure 3. 11 Principles of serum triglyceride

3.11.2 Reagents: R1: 150000 U/l lipoprotein lipase, 800 U/l glycerol kinase, 4000 U/l glycerol-3-P-oxidase, 440 U/l Peroxidase, 0.7 mmol/l 4-Aminoantipyrine, 0.3 mmol/l ATP and 7.5 mmol/l phenol

R2: Glycerol equivalent to a concentration of 200 mg/dl (2.28 mmol/l) triglycerides.

3.11.3 Procedure*:* To labeled test tubes, 1.0 ml of reagent R1 was mixed with 10 µl of patient serum sample or 10 µl of glycerol standard (R2). After 5 minutes of incubation at 370C, the absorbance was measured at 500 nm against the reagent blank.

Calculation: Triglyceride concentration in the samples was calculated against the absorbance of the standard as follows:

Triglycerides (mg/dl) = (A sample / A standard) X Concentration of the standard,

Where A=absorbance at 500 nm

Table 3. 11 Risk stratification of serum triglyceride concentrations

Triglyceride Concentration(mg/dl)	Risk Classification
<150	Normal
150-199	Borderline
200-499	High
≥500	Very high

3.12 Determination of Serum HDL-cholesterol Level

HDL-cholesterol in the patient‘s blood samples was estimated by using a commercial kit, based on the fact that Apoipoproteins B-containing lipids, namely chylomicrons, LDL and VLDL can be selectively precipitated from serum using phosphotungstic acid, leaving HDL in the supernatant obtained from Cromatest®-Cholesterol MR, Linear Chemicals SL, Barcelona, Spain[53].

3.12.1 Principle: Chylomicrons, VLDL and LDL are precipitated with phosphotungstic acid and magnesium chloride and removed by centrifugation. The supernatant contains HDL only as a significant source of cholesterol, and was used for estimating cholesterol by using a kit as mentioned above for total cholesterol determination.

3.11.2 Reagents and Procedure: Precipitating reagent: made from four parts by volume of 0.55 mmol/L phosphotungstic acid, 25 mmol/L MgCl2, plus one part by volume of deionized water.

Assay Reagent: 200 mmol/L PIPES pH 7.0, containing 1 mmol/L sodium cholate, > 250 U/L cholesterol esterase, > 250 U/L cholesterol oxidase, >1 KU/L peroxidase, 0.33 mmol/L 4-aminoantipyrine, 4 mmol/L phenol and 2 g/L non-ionic surfactant, plus unspecified biocides.

First, the precipitating reagent (500 uL) was mixed with 200 µL of serum and incubated for 10 minutes at room temperature. The incubated mixture was centrifuged for 10 minutes at 4000 g and the clear supernatant, containing the HDL, was separated from the precipitate. To labeled test tubes, 1.0 ml of the Assay Reagent was mixed with 10 µl of clear supernatant containing the HDL. After 5 minutes of incubation at 370C, the absorbance of the sample and standard HDL solutions were measured at 546 nm against the reagent blank.

Table 3. 12 Desirable serum HDL-cholesterol concentrations for men and women.

Men: HDL > 40 mg/ dL
Women: HDL > 50 mg/dL

3.13 Determination of Serum LDL-cholesterol Level

A method developed by Friedwald‘s is used to estimate LDL-C level in the [54]. The method involves measurements of fasting total cholesterol, triglyceride, and high-density lipoprotein

cholesterol concentrations and calculating the value of LDL-c by using Friedwald‘s formula. It may not reliable at very low LDL cholesterol concentrations and /or very high (>400 mg/dl) triglyceride values.

In Friedwald‘s formula, TG replaces VLDL, because it has been shown that serum TG levels are equivalent to five times the levels of VLDL.

Friedwald‘s formula:

LDL-C = Total Cholesterol – [HDL-C + (TG/5)]

For all diabetics, the recommended serum LDL-c level is less than 100 mg/dL, though if a diabetic has known coronary artery disease the goal is even more stringent, at 70 mg /dL or lower. None of the patients in this study had known coronary artery disease, so optimal LDL-c levels for all patients studied is 100 mg/dL or less [54].

3.14 Determination of Serum Apo lipoprotein A-I (ApoA-I) and Apo protein B Levels

Measurement of serum ApoA-I and ApoB levels are useful in predicting patients with high risk of coronary artery disease (CAD). Levels of ApoA-I are inversely correlated with the risk of premature CAD. The relative proportion of ApoB, a major component of VLDL and LDL, to ApoA is effective in differentiating individuals with or without ischemic heart disease. An increased ApoB/ApoA-I ratio at a young age is potentially a marker for CAD.

3.14.1 Principle and Procedure: The ApoA-I and ApoB assays area immunoturbidimetric procedures that measures increasing sample turbidity caused by the formation of insoluble immune complexes when either antibody to ApoA-I or antibody to ApoB is added to the sample. Serum samples are incubated in a buffer containing anti-ApoA-I antibody or anti-ApoB-antibody in excess, which comes as a standard assay kit (obtained from Abbot Chemical Company, USA). In the presence of an appropriate antibody in excess, the ApoA-I or ApoB precipitates and its concentration is measured as a function of turbidity. After incubation of serum samples with kit reagents at room temperature for 20 minutes, turbidity of samples were measured at 340 nm against a reagent blank. Turbidity is proportional to the concentration of the Apoipoproteins being measured: the kit is already standardized against Apo lipoprotein A-I and Apoipoproteins B standard concentrations. According to the manufacturer‘s data, a normal Apo lipoprotein A-1 is less than 176 mg/dL and normal Apo lipoprotein B levels are below 114 mg/dL.

3.15 Determination of Serum Insulin Concentrations

3.15.1 Principle and Procedure: Insulin was measured using Elecsys2010 immunoassay analyzer, using an Insulin Reagent kit from Roche Company. The Elecsys2010 analyzer is a fully automatic run-oriented analyzer system for the determination of immunological test using anElectrochemiluminescence immunoassay –ECLIA–process. In this assay, all components and reagents for routine analysis are integrated into the analyzer, so the process is fully automated and the insulin concentrations are determined by the analyzer. The assay is based on initial binding to insulin (in the serum sample) of a monoclonal anti-insulin antibody that is conjugated to biotin. The insulin-antibody-biotin complex then binds, via biotin residues, to a second antibody against the first anti-insulin antibody.

The second antibody is conjugated to a Ruthenium 2+ complex. Quantitation of the Ruthenium 2+ is achieved through an electrochemical reaction that is linked to a light-generating reaction. The chemiluminescence intensity generated in the final step is related to the insulin concentration in the serum sample: this is automatically determined by the analyzer.

3.15.2. Factor affecting serum insulin

- Substances that alter the measurable concentration of the analyte or alter antibody binding can potentially result in immunoassay interference.
- Interfering, endogenous substances that are natural, polyreactive antibodies or autoantibodies (heterophiles), or human anti-animal antibodies together with other unsuspected binding proteins that are unique to the individual, can interfere with the reaction between analyte and reagent antibodies in immunoassay.
- Lipaemia, cross-reactivity, and exogenous interferences due to pre-analytical variation, matrix and equipment reaction also affect immunoassay.
- Interfering substances may lead to falsely elevated or falsely low analyte concentration in one or more assay systems depending on the site of the interference in the reaction and possibly result in discordant results for other analytes.

Reference (normal values) range for serum insulin using this kit: 2.6-24.9 (µU/mL)

3.16 Calculation of HOMA-IR Index

HOMA-IR values are an estimation of insulin resistance/ sensitivity and are widely used in clinical research to assess insulin sensitivity. Normal adult subjects have a HOMA-IR cutoff of >2.5. HOMA-IR was calculated from fasting glucose and insulin concentration by using the following formula [55, 56].

$$\textbf{HOMA-IR} = \frac{\{\text{Glucose in mmol/L}\} \times \{\text{Insulin in } \mu\text{U/mL}\}}{22.5}$$

The 22.5 comes from the product of a normal fasting plasma insulin (5 µU/mL) multiplied by a normal fasting glucose (4.5 mmol/L), and allows the patient‘s values to be normalized.

A normal HOMA-IR in this study was taken to be 3.0, which is in the range often used, though normal cut-offs vary somewhat. A HOMA-IR of less than 3 were considered to represent insulin sensitivity, whereas a HOMA-IR of 3 or above means that a person has insulin resistance.

3.17 Smoking and Framingham Risk Scores

Of the 106 diabetics, 16 were cigarette smokers. Diabetics who smoke are at a significantly increased risk of cardiovascular disease compared with diabetic non-smokers. The Framingham risk score uses the following parameters: sex (male or female), age, smoking status (yes or no), systolic blood pressure (in mm Hg), use of blood pressure medications (yes or no) were calculated for smokers only, serum triglyceride (in mg/dL) and serum total cholesterol (in mg/dL) to calculate the percentage risk of a person suffering from a myocardial infarction during the next ten years. It is based on a study of tens of thousands of individuals over many decades in the USA, but can be generally applied to other populations [57].

The risk is based on a complex equation, and can be determined by inserting the patients‘ parameters into an online site; the risk factor is then determined automatically [58].

Framingham scores were determined for smokers only, and were also calculated to show what the risk would be if the patient was not a smoker. Only 10 of the 16 smokers studied could have their Framingham score evaluated because the score works only for LDL within the range 130 to 320 mg/dL and 6 of the 16 patients had LDL levels outside of this range. Performing Framingham scores with smokers and comparing them with the situation if they did not smoke allows evaluation of the contribution of smoking to the patient‘s cardiovascular risk [58].

3.18 Statistical analysis

Information obtained from questionnaire and laboratory analyses were analyzed using the computer statistics SPSS version20 package. In all cases, a p-value of 0.05 or lower was considered statistically significant. Values are expressed as mean plus or minus SEM. To check statistically significant difference between the variables in the two groups, the T-test was used. Linear regression analysis (Pearson correlation test) was used to evaluate association between the variables.

CHAPTER 4. RESULTS

4.1 Demographic characteristics of diabetic patients

There were more females (55.7%) than males (44.3%), and larger proportion of patients were between 41 and 50 years old. Patients older than 55 were not included in the study because data shows that beyond the mid-fifties HOMA-IR is not a reliable measure of insulin resistance, especially in women [55]. Over a half (about 60%) of patients were overweight or obese; obesity was less prevalent than overweight (44.3 % were overweight, 16 % were obese). About 40% of patients were normal weight (37.7%). A family history of diabetes in first degree relatives was found in 38 (35.8 %) of the study subjects (Table 4.1).

Table 4.1: Demographic characteristics of diabetic patients in the study

Variable		Number (N)	Percentage (%)
Sex	**Male**	47	44.8 %
	Female	59	55.2 %
Age	**30**	3	2.8 %
	31 to 40	23	21.7 %
	41 to 50	61	57.5 %
	51 to 55	19	17.9 %
BMI	**< 18.5**	2	1.9 %
	18.5 to 24.9	40	37.7 %
	25 to 29.9	47	44.3 %
	30 and over	17	16 %
Family History	**yes**	38	35.8 %
Diabetes *	**No**	68	64.2 %

*Family history refers to having a first degree relative with diabetes

The present study was carried out on type 2 diabetic patients, age range 30-55 years old, with a median age 46 (range 25), attending the outpatient Diabetic Clinic of TASH. For blood sugar determination, one patient was eliminated because of a very low blood glucose level (26 mg/dL) that was thought to be erroneous, leaving 105 patients.

It was found (Table 4.2) that most of the studied patients (84/105 or 80 %) had poorly controlled diabetes (fasting blood glucose greater than 126 mg/dL) based on their fasting blood glucose. Only 21 patients (20%) had fasting blood glucose levels below 126 mg/dL and were considered to be well controlled diabetics.

Of the 105 diabetics, 47 (44.8) were male and 58 (55.2) were female subjects. The percentage of females with poor glycemic control (47/58, 81 %) was similar to the percentage of males who had poor glycemic control (37/47, 79 %).

Table 4.2: Fasting blood glucose (FBG) levels in 105 patients with type 2 diabetes. (N = number of patients).

Fasting blood glucose (FBG)	All patients N (%)	Male patients N (%)	Female patients N (%)
Normal FBG(<126 mg/dL)	21 (20%)	10 (21%)	11 (19%)
High (FBG > 126 mg/Dl)	84 (80%)	37 (79%)	47 (81%)
Total	105	47(44.7%)	58(55.2%)

4.2 Levels of Total Cholesterol, LDL-c, HDL-c and Triglycerides

One of the diabetics had a TG over 400 mg/dL and was an outlier; whether this was a true value or not is unclear, but this patient was eliminated from the statistical calculations. Another patient had an LDL less than 5 m/dL and this is too low to be valid in the Friedwald‘s equation and probably an erroneous value, so this patient was also eliminated from statistical calculations of cholesterol values.

Of the 104 diabetics studied, 63 (61%) had a normal serum total cholesterol, below 200mg/dL, whereas 41 (39%) had abnormal total cholesterol levels, above 200 mg/dL., 43 out of 104 diabetic patients had showed normal Triglyceride levels (<150 mg/dL) whereas the remaining diabetic patients 61 (58.7 %) showed abnormally high TG levels.

52(50 %) Of the 104 diabetic patients had an LDL-c level below 100 mg /dL. Whereas the remaining 52(50 %) diabetic patients had an abnormal LDL-c level (over 100 mg /dL For HDL-c levels, only 24/104 (23.1 %) of patients had normal cholesterol level. Whereas the remaining diabetic patients had, 80/104 (76.9 %), had abnormally low levels of serum HDL-c.

Almost a half of all patients were taking a statin and, of these, only about a half of them had controlled LDL-c levels (less than 100 mg/dL). About a half of patients not on a statin also had elevated LDL-c levels (> 100 mg/dL) and so overall about a half of the diabetics did not have good control of their LDL-c.

Table 4.3: Number of diabetic patients with normal and abnormal serum levels of Total cholesterol, Triglycerides, LDL-c and HDL-c

Parameter	Serum level	N (%)
Total Cholesterol	Normal (< 200 mg/dL)	63 (61%)
	High (> 200 mg/dL)	41 (39%)
Triglyceride	Normal (< 150) mg/dL	43 (41.3%)
	Elevated (> 50) mg/dL)	61 (58.7%)
LDL cholesterol	Normal (< 100 mg/dL)	52 (50%)
	Elevated(>100 mg/dL)	52 (50%)
HDL cholesterol	Normal*	24 (23.1)
	Low*	80 (76.9%)

***Normal HDL-c is > 40 mg/dL for men and > 50 mg/dL for**

4.3 Results of measure biochemical parameters in diabetic patients

Mean values of various biochemical parameters measured in this study, comparing diabetics with well controlled fasting blood glucose (less than 126 mg/dL) to those with poorly controlled blood glucose (126 mg/dL or above) are summarized in Table 4.4. It can be seen that total cholesterol, Triglycerides, LDL-c, HDL-c and HOMA-IR indices showed statistically significant differences between diabetics with good and those with poor glycemic control. There were no significant differences between serum insulin levels, Apo lipoprotein A-1 and Apo lipoprotein B levels between controlled and uncontrolled diabetics.

The mean blood cholesterollevel for all type 2 diabetic patients (104) was found to be 188±65. Most of the diabetic patients with poor diabetic controls (89/104) had blood cholesterol level of 197±63 mg/ml. whereas the remaining diabetic patients (15/104) with good diabetic control have blood cholesterol level of 137±57. Poorly controlled diabetic patients had significantly elevated level of cholesterol ($p<0.001$) as compared with the good controlled diabetes.

The mean triglyceride, LDL and HDL of type 2 diabetic patients (104) were (189±114.7, 113.5±53.5 and 35.2±11.9), respectively. Most of the diabetic patients with poor controlled diabetes showed high level of TG,LDLand low level of HDL (201 ± 116.6,118 ± 53 and 36.3 ± 11.5) respectively. Whereas, the remaining diabetic patients with good controlled diabetes showed normal levels of TG, LDL and HDL (120 ± 73,83 ± 41 and 28.5 ± 12.5),respectively.Poorly controlled diabetic patients had significantly elevated level of TG, LDL ($p<0.05$) as compared with the good controlled diabetes. Furthermore, poorly controlled diabetic patients had significantly lowered HDL as compared with the good controlled diabetes.

The mean serum insulin level of diabetic patient‘s was21.7 ± 17.1. Most of the poor controlled diabetic patients had a mean serum insulin level of 22±28. Whereas the remaining diabetic patients with good controlled diabetes has a mean insulin level of 18.7±8. Serum insulin levels between poor and good controlled diabetes did not show significant differences.

The mean HOMA-IR of the diabetic patient‘s was10.6 ± 9.Poor controlled diabetes (89/104) hadmean HOMA-IR values of 11.7 ± 9. Whereas the mean HOMA-IR for good controlled diabests was 4 ± 2.6. There was a significant difference ($p<0.05$) in the mean values of HOMA-IR among the poor and good controlled diabetes,

Table 4.4: Mean values of various biochemical parameters of diabetic patients studied.

Parameter studied	All patients (N = 104)	Poorly controlled diabetics (N = 89)	Well controlled diabetics (N = 15)	p-value
Total cholesterol [1]	188 ± 65	197 ± 63	137 ± 58	0.001
Triglyceride [1]	189.6± 114.7	201 ± 116.6	120 ± 73	0.010
LDL-c [1]	113.5 ± 53.5	118 ± 53	83 ± 41	0.017
HDL-c [1]	35.2 ± 11.9	36.3 ± 11.5	28.5 ± 12.5	0.019
Serum insulin [2]	21.7 ± 17.1	22 ± 18	18.7 ± 8	0.47
HOMA-IR	10.6 ± 9	11.7 ± 9	4 ± 2.6	0.002
Apo lipoprotein A-1[3]	66.3 ± 32.3	61 ± 24.7	67 ± 33	0.36
Apo lipoprotein B [3]	20.6 ± 15.3	21 + 16	16 ± 7.6	0.22

(P-value refers to comparison of values for poorly controlled with controlled diabetics [1] Units in mg/dL, ([2] Units in μU/mL, ([3] Units in mg/dL.)

Table 4.5 Number of diabetic patients with normal serum LDL-c or high serum LDL-c levels taking a statin or not taking a statin.

Statin status:	LDL-c normal (<100 mg/dL)	LDL-c high (>100 mg/dL)	Total
Patients on a statin	24 (51%)	23 (49%)	47
Patients not on a satin	28 (49%)	30 (51%)	58

4.4Insulin levels and HOMA-IR values in diabetic patients

As shown in Table 4.4, of the 105 patients with meaningful blood glucose levels, 73 (69.5 %) of them had normal serum insulin levels, and 32 (30.5%) had elevated insulin levels. There was no statistically significant difference in serum insulin levels between the poorly controlled (insulin levels 22 ± 18) and uncontrolled (18.7 ± 8) diabetics (p = 0.453).

Of the 105 patients, only 12 patients (11.4%) had a normal HOMA-IR value, whereas most patients (93/105, 88.6 %) had high HOMA-IR values, indicating that they were insulin resistant (Table 4.6). HOMA-IR, a measure of insulin-sensitivity and resistance, showed significant ($p = 0.01$) differences between poorly controlled diabetics (11.7 ± 9) and controlled diabetics (4.0 ± 2.6).

This indicates that poorly controlled diabetics have higher insulin resistance than well controlled diabetics. Of the 12 patients with normal HOMA-IR values, 8 had controlled diabetes and only 4 had uncontrolled diabetes. This means that 8/21 (38 %) patients with controlled patients had normal HOMA-IR, whereas only 4/84 (5 %) patients with uncontrolled diabetes had normal HOMA-IR.

Table 4.6: Number and percentage of diabetic patients with normal and elevated Serum insulin levels and HOMA-IR values. (N = number of patients)

Parameter		N (%)
Insulin (serum)	**Normal (2.6 – 24.9 µL/mL)**	73 (69.5%)
	High (> 24.9 µL)	32 (30.5%)
HOMA-IR	**Normal (<3.5)**	12 (11.4%)
	High (>3.5)	93 (88.6%)

To determine the effect of medication treatment on insulin levels and HOMA-IR, the values of these parameters were determined for patients taking glibenclamide alone, metformin alone and both glibenclamide PLUS metformin. Glibenclamide is a sulphonylurea and stimulates insulin secretion by acting on pancreatic beta cells, whereas metformin works in multiple ways to reduce hepatic gluconeogenesis and glucose release as well as to improve insulin sensitivity of peripheral tissues [59, 60, and 61].

From Table 4.7, it can be seen that HOMA-IR indices were significantly lower (p=0.001) in diabetics treated with metformin, compared with glibenclamide alone. This agrees with the known ability of metformin to improve peripheral insulin resistance. Table 4.8 shows also that glibenclamide, as sulphonylurea, significantly increased serum insulin levels, in agreement in the known mechanism of sulphonylurea as stimulators of pancreatic beta-cell insulin secretion.

Table 4.7 Mean HOMA-IR indices for patients on metformin alone, glibenclamide alone, and metformin *plus* glibenclamide.

Metformin only (N=54)	**Glibenclamide only (N=15)**	**Metformin PLUS Glibenclamide (N = 36)**	**p-value**
26.8 ± 20.6	32.5 ± 15.6	15.3 ± 11.9	0.001

Table 4.8 Mean serum insulin levels (µU/mL) in diabetics on metformin alone, glibenclamide alone, and metformin *plus* glibenclamide

Metformin only (N=54)	**Glibenclamide only (N=15)**	**Metformin PLUS Glibenclamide (N = 36)**	**p-value**
8.5 ± 8.3	15.4 ± 11.4	11.8 ± 8.2	0.019

4.5Serum Apo lipoprotein A-1 and Apo lipoprotein B in patients.

All diabetic patients studied had normal values of serum Apo lipoprotein A-I (ApoA-1) 106 patients examined) Apo lipoprotein B (ApoB) of 105 patients examined that were within the normal reference range given by the company that supplied the test kits (Table 4.4), except for one patient that had a very high (1000 mg/dL, normal less than 125 mg/dL) Apo lipoprotein B level. This isolated outlier for ApoB was not included in statistical data and was probably erroneous. There were no abnormal values for these Apolipoprotein levels otherwise. There were no statistically significant differences between diabetics with good glycemic control and those with poor glycemic control with respect to serum apoA-1 or serum ApoB levels.

4.6Blood Pressure Control in Diabetics

Most (85/105, 81 %) of the diabetic patients had well controlled systolic blood pressure (SBP < 140 mmHg). Similarly, diastolic blood pressure was well controlled (< 90 mm Hg) in most of the diabetic patients (80/105, 76 %) patients. *Both* diastolic and systolic blood pressure together were well controlled in 72 /105 (69 %) of patients studied.

4.7BMI, waist circumference and Insulin Resistance

A substantial proportion of the diabetics, 41/105 (39%), were either underweight or normal weight (BMI less than 25). Of these, most of them were normal weight, and most of these were also insulin-resistant (high HOMA-IR). The remaining (64) 61 % of diabetics were overweight or obese (BMI 25 or over) and although waist circumference increased as BMI increased, a substantial number of patientswith a normal BMI had a high waist circumference.

To test this idea further, the actual numbers of males and females with high waist circumferences who had BMIs below 25 or above 25 were determined. This showed that 11 Males with a normal BMI had an abnormal waist circumference (over 40 inches), and 12 males with normal BMIs had a normal waist circumference (under 40 inches). Most males (20/24) who were overweight (BMI 25 or over) or obese (BMI 30 or over) also had an abnormally high waist circumference. For females, most who had a normal BMI (17/22) had a high waist circumference, and most of those who had a BMI of 25 or over (37/40) had a high waist circumference. This suggests that there are sub-populations of men and women who have relatively high abdominal obesity and subpopulations who have no abdominal obesity. Almost all patients with a normal BMI, whether male or female, had an elevated HOMA-IR, suggesting that they had significant insulin resistance despite their normal weight. This will be discussed in the Discussion section in relation to the possibility that there are different groups of type 2 diabetics in this population of Ethiopian patients

4.8Framingham scores and smoking status of diabetic patients

There were 16 cigarette smokers (15%) among the 106 diabetic patients studied, and 2 of these were female, 14 were male. Of these, Framingham risk scores could be determined. The Framingham risk score is based on a person‘s sex, age, systolic blood pressure (or use of anti-hypertensive), total cholesterol, triglyceride and smoking status, and limits the percentage chance a person will have a first heart attack in the next 10 years. It is based on prospective epidemiological data on thousands of US citizens and is fairly reliable even in other populations. The Framingham risk score was calculated using an online site in which the patient‘s parameters are inserted into a program [49].

That calculates the risk score. Table 4.9 shows Framingham risk scores for the 10 diabetics. It can be seen that smokers in their 30s had a low risk (1%) of having a heart attack in the next 10 years, whereas patients in their 40s and 50s had a much higher risk (8 to 22%). When Framingham scores were determined as if the patients were non-smokers, the risk decreased significantly compared with the patient being a smoker: for example, several patients with risk scores of 8 to 18 % would have significantly lower risk, from 2 to 8 %, of having a heart attack if they were non-smokers. Another smoker had a risk score of 22%, which decreased to 13 % if he was not a smoker. This emphasizes the need for diabetics to be counseled about smoking cessation or abstinence.

Table 4.9 Framingham Risk Score for 10 smokers in the diabetic population under study.

Age	Gender	Total cholesterol	HDL cholesterol	SBP (mmHg)	Onblood pressure medication?	Framingham score as a smoker	Framingham score if patient was a non-smoker
39	F	177 mg/dl	34 mg/dl	100	No	1 %	1%
35	F	177 mg/dl	52 mg/dl	140	No	1 %	1%
50	M	190 mg/dl	51 mg/dl	100	No	8 %	4 %
52	M	245 mg/dl	37 mg/dl	110	No	18 %	8 %
50	M	252 mg/dl	45 mg/dl	120	No	9 %	7 %
42	M	287mg/dl	49mg/dl	100	No	14 %	4 %
47	M	262mg/dl	54mg/dl	140	No	18 %	4 %
46	M	181mg/dl	37mg/dl	110	No	8 %	2 %
50	M	221mg/dl	24mg/dl	120	No	22 %	13 %
50	M	168mg/dl	29mg/dl	110	No	10 %	4 %

CHAPTER 5: DISCUSSION

In this study, 106 diabetics attending the Tikur Anbessa Hospital Diabetes Clinic were chosen who were not taking insulin and were classified as type 2 diabetics. Their fasting blood glucose concentrations, fasting serum total cholesterol, triglyceride, LDL-cholesterol, HDL-cholesterol, fasting serum insulin, Apo lipoprotein A-1 and Apo lipoprotein B were measured biochemically, their HOMA-IR indices were calculated, and blood pressure, waist circumference and BMI were determined.

5.1 Glycemic control in diabetics at Tikur Anbessa Hospital Diabetes Clinic

It was found in this study that most of the diabetic patients (80%) had poorly controlled fasting blood glucose levels: only one in five patients had well controlled blood glucose (Table 4.2). The study did not evaluate possible reasons why there was such a high level of poorly controlled diabetics in this outpatient hospital populations. Possibilities include poor adherence to medications patients, poor follow-up with their doctor, lack of adequate education regarding the importance of checking their blood sugars regularly and achieving proper diabetes control, inadequate medication and patient education by the doctor and healthcare staff involved, unpleasant side-effects of medications, and restrictive financial costs of diabetes medications and care.

Other studies of diabetes patients in various areas of Ethiopia also have shown that many, if not most, diagnosed diabetic patients have inadequately controlled diabetes.Examined 384 diabetic patients in Jimma Specialized Hospital Diabetic Clinic and found that only 41.8% of patients had adequately controlled glycemic control; 58.2% of diabetics were not adequately controlled [62].

Inability to pay for medications, lack of knowledge about the importance of diabetic control, perceived side-effects, non-adherence with medications and failure to disclose non-adherence with their doctor, were major reason why these patients had poor diabetic control.

In a study of 391 diabetic patients in the Gondar University Hospital Diabetic Clinic, 65% of patients had poor glycemic control (HbA1c equal to or over 7.0), and over a half of patients did not take their medications properly. Taking insulin, using traditional medical practitioners and dissatisfaction with their care were associated with poor medication adherence [63]. In another study of diabetic patients in Addis Ababa, 58.8 % of diabetics had poor glycemic control (FBG over 126 mg/dL) and poor dietary practice and nutrition knowledge, concerns about food costs, despondency, and poor education from healthcare professionals were common [64,65]. Also found that diabetic patients in the Adama Referral Hospital diabetic clinic generally had poor glycemic control, particularly due to poor adherence to medications and lack of adequate knowledge and practice of self-management of their diabetes[66].Studied 222 diabetics from three hospitals in Harar town, Ethiopia, and found that only 39.2% were following the recommended care for their diabetes. Diabetics with a low income and high education level were more likely to follow diabetic self-care properly.

Diabetic patients in a Mekelle hospital had generally poor control diabetes control: 68% had a HbA1c over 10 %. Micro vascular complications were common: retinopathy 21%, neuropathy 41%, advanced nephropathy 2%, but 51% had micro albuminuria, a sign of early diabetic kidney damage. About 6 % had peripheral vascular disease and none had known coronary artery disease. Only 2% were smokers, and lipid profiles and blood pressure were generally good.

Diabetics taking insulin tend to have poor glycemic control compared with diabetics taking oral medications alone [67].Found that 81.7% of insulin-treated diabetics in Jimma University Hospital had poor glycemic control and most (83%) of these diabetics had one or more diabetic complications. Non-adherence to insulin regimens and diet, poor blood sugar monitoring, and lack of knowledge about symptoms of hyperglycemia were among the main reasons for poor glycemic control [68].Examined the knowledge and practices of 410 diabetics in a hospital in Northwest Ethiopia. About a half of the patients had good knowledge about diabetes, but only about a third of patients practiced proper care of their diabetes. Younger age, higher educational status, increased income level and increased duration of diabetic therapy were associated with good knowledge and practice.

These studies show that diabetes is poorly controlled in many known diabetics in Ethiopia, and that diabetic complications are also common, and they stress the importance of education of diabetic patients, in particularly about taking their medications properly, adhering to a healthy diet and following recommendations from healthcare professionals on self-care of their diabetes. In addition [69].Found that there was a 125% increase in both type 1 and type 2 diabetes in patients attending the outpatient department of Gondar University Teaching Referral Hospital between 2000 and 2009. The mean body mass (BMI) of diabetics also increased significantly during this time period, indicating the need for dietary and other preventive lifestyle education in Ethiopia.

Diabetic patients in a Black lion hospital had most of the patients: 70.8 % were found to have poor glycemic control, i.e., were having HbA1c more than 8.0 g %. Poor glycemic control was more prevalent in the patients managed by single drug therapy (75 and 71.5 % in patients on insulin or glibenclamide, respectively) compared to those maintained on metformin plus glibenclamide (54.5 %) [29].

Therefore this study generally agrees with numerous other studies of diabetics in Ethiopia, that glycemic control is not achieved by many diabetics, putting patients at risk of developing serious macrovascular and micro vascular diabetic complications. It emphasizes the need for better education of diabetics and better healthcare provisions and access for proper management of diabetes.

5.2 Cholesterol profiles and use of statin in diabetic patients at TASH

The results show that LDL-c, a major risk factor for cardiovascular disease, was greater than 100 mg/dL, that is, abnormally high for a diabetic, in a half of all diabetics studied. The other half had well controlled LDL-c (Table 4.5). Statins are used mainly to treat elevated LDL-c, and are widely used in diabetics. It has been recommended that all diabetics over 40 take a statin, whether or not their LDL-c is at goal, because statins have beneficial effects in addition to their ability to improve cholesterol levels; for example, statins reduce inflammation and atherosclerotic plaque development [70]. Also, about a half of all patients that were not on a statin had LDL-c levels above 100 mg/dL, and only 50 % of patients taking a statin had adequate control of their LDL-c (Table 9). These data suggest that statins need to be used more frequently and perhaps at higher doses for some patients, in this diabetic population.

HDL-c levels were abnormally low in more than three-quarters of the diabetics (Table 4.3). Low HDL predisposes to cardiovascular disease and is difficult to treat, whereas elevated HDL-c protects against cardiovascular disease [71, 72]. HDL-c was statistically slightly worse (lower) in diabetics with good glycemic control compared to diabetics with poor glycemic control, whereas LDL-c was better in glycemically controlled patients compared with those with poor glycemic control (Table 4.4).

Triglyceride levels were statistically better in controlled, compared with uncontrolled, diabetics (Table 4.4), which is not surprising considering that elevated triglycerides and low HDL-c occur in diabetic dyslipidemia, which usually improves with better glycemic control.

5.3 Serum insulin and insulin resistance (HOMA-IR)

Serum insulin levels did not differ statistically between diabetics with good or bad glycemic control (Table 4.4). However, there was a statistically significant difference in insulin sensitivity, as measured by HOMA-IR, between glycemically controlled and uncontrolled patients (Table 4.4). Patients with good control of their diabetes had lower HOMA-IR indices. Most of the diabetics studied were poorly controlled with respect to their blood glucose, but 12 patients did have a normal HOMA-IR, and of these 12 patients, 8 had well controlled diabetes. Metformin improves insulin resistance in diabetic patients [73, 74]. And this may be why the controlled patients had lower HOMA-IR indices. In support of this, metformin-treated patients had lower HOMA-IR values than glibenclamide-treated patients, suggesting that metformin did lower HOMA-IR in these patients (Table 4.7).

Glibenclamide, a sulphonylurea, does not lower insulin resistance, but it works by increasing secretion of insulin from pancreatic beta cells [75]. In support of this, glibenclamide-treated patients had significantly higher serum insulin levels compared with patients treated with metformin alone (Table 4.8). Metformin does not raise serum insulin levels.

5.4 HOMA-IR indices in relation to waist circumference and BMI

The diabetic population studied here consisted of a significant number of patients (41/105 or 39%) who were normal weight or underweight (BMI<25), though most (61%) were overweight or obese. However, there appeared to be two groups of these non-overweight diabetics: one group had an abnormally high waist circumference, and another group had a low waist circumference. A half of males with a normal BMI had a relatively high waist circumference, while a half had a low waist circumference. Most females with a normal BMI also had a high waist circumference. This suggests that there may be two groups of ―no obese" (normal BMI) diabetics: one group with abdominal (visceral) obesity but with a normal overall BMI and another group with no excess abdominal obesity and having a normal BMI. This finding may provide some insights into ―thin" type 2 diabetics, or ―metabolically unhealthy, normal weight individuals," who appear to have type 2 diabetes in the absence of being obese or overweight [7]

5.5 Blood pressure control and smoking status

In the study reported in this thesis, blood pressure was well controlled in over two-thirds of patients, 15% (16/106) were smokers (14 males and 2 females) and none had known cardiovascular disease. The Framingham risk scores that were calculated for some of the smokers (Table 14) showed that some of them, especially those in the 40s and older, had significantly elevated risk for having a heart attack in the next 10 years. When Framingham scores were calculated for these smokers as if they were non-smokers, significant reductions in risk of myocardial infarction were seen. This reinforces the argument that smoking cessation in diabetics needs to be assessed and that diabetic patients who smoke need to be encouraged to quit smoking, and prevention of smoking should be part of a public health programme.Smoking tends to increase blood cholesterol levels. Furthermore, the ratio of high-density lipoprotein (the "good" cholesterol) to low-density lipoprotein (the "bad" cholesterol) tends to be lower in smokers compared to non-smokers. Smoking also raises the levels of fibrinogen and increases platelet production (both involved in blood clotting) which makes the blood viscous. Carbon

monoxide binds to hemoglobin (the oxygen-carrying component in red blood cells), resulting in a much stable complex than hemoglobin bound with oxygen or carbon dioxide—the result is permanent loss of blood cell functionality. Blood cells are naturally recycled after a certain period of time, allowing for the creation of new, functional erythrocytes. Carbon monoxide exposure reaches a certain point before they can be recycled, hypoxia (and later death) occurs. All these factors make smokers more at risk of developing various forms of arteriosclerosis. As the arteriosclerosis progresses, blood flows less easily through rigid and narrowed blood vessels, making the blood more likely to form a thrombosis (clot). Sudden blockage of a blood vessel may lead to an infarction (stroke). Smoking also increasesblood pressure and weakens blood vessels

5.6 Apo lipoprotein A-1, Apo lipoprotein B and ApoB: ApoA-1 ratio

Almost all of the patients in this study had normal serum Apo lipoprotein A-1 and Apo lipoprotein B levels, and levels of these Apo lipoproteins did not differ significantly between diabetics with well controlled and poorly controlled blood glucose. There was also no correlation between ApoB: ApoA-1 ratios and diabetic control. It is unclear what these result mean. There could have been an experimental technical problem, causing the results to be erroneous; or the range of normal values given by the manufacturer may not apply to this population; or it could be that these patients genuinely did have normal serum ApoA-1 and ApoB levels.

CHAPTER 6: CONCLUSIONS

The morbidity and mortality in DM are related to its macro- and micro vascular complication. Insulin resistance and dyslipidemia play their role in the pathogenesis of these complications. These patients have a dyslipidemic profile consisting of high serum levels of cholesterol, triglycerides and low serum levels of high density lipoprotein, which play a significant role in acceleration of atherosclerosis in patients with DM. There is high incidence of poor glycemic control in Type 2

Diabetic patients attending the diabetic clinic of Tikur Anbessa Specialized Hospital, Addis Ababa University, Addis Ababa. Poor glycemic control seems to be related to diabetic dyslipidemia, and many of the patents in this study had uncontrolled dyslipidemia.

CHAPTER 7: LIMITATIONS

This study was a cross-sectional study of 106 diabetic patients at Tikur Anbessa Hospital Diabetic Clinic. This is a relatively small number of patients studied, so the statistical strength may be limited. Further studies should be done with greater numbers of patients and HgA1c test because the test is very expensive. Also, the study excluded type I diabetics and any type 2 diabetics taking insulin. This was done to remove any concerns about exogenous insulin interfering with serum insulin assays, since the serum insulin is crucial in measuring HOMA-IR. Nevertheless, it will be of interest to examine type 1 diabetics for glycemic control as well as for dyslipidemia and other parameters such as blood pressure, waist circumference, BMI. Furthermore, patients were not asked questions about their knowledge of diabetes or about dietary and other lifestyle management and their knowledge of risks and types of complications they may get from uncontrolled diabetes.

CHAPTER 8: RECOMMENDATIONS

The study led to a new hypothesis, that there may be subclasses of type 2 diabetes in this population, because a substantial number of the diabetics were normal weight or underweight, which is a different pattern to that seen in western patients, where the majority of diabetics are overweight or obese. Some of the normal-BMI patients had relatively high waist circumferences, and some had normal or low waist circumferences. This might suggest that there are type 2 diabetics with normal BMI: ones with abdominal obesity and one without.

This can be further tested with more patients and with more accurate ways of determining body fat. There is some evidence to suggest that chronic excess sucrose intake can cause diabetes in the absence of causing excess accumulation of body fat, producing ―thin" diabetics. Sugar intake was not assessed in these patients, but it could be incorporated into a future.

HOMA-is differ between different populations and ethnic groups, though the differences are not very large. A cut-off value of 3.0 was used to define the boundary between insulin resistance and insulin sensitivity, but this may not be entirely accurate because we do not have the normal values for Ethiopian populations.

Clearly, better diabetic education is needed for many diabetic patients in order to improve diabetes control and reduce the risk of diabetic complications, and education aimed at encouraging smoking prevention and cessation is essential to further reduce cardiovascular risks among smoking diabetics

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Annex I: Information sheet for participants

Information sheet for participants of the study entitled insulin resistance in type2 diabetic patients

Information sheet (English Version)

Addis Ababa University College of health science department of clinical laboratory

Principal Investigator: Betelhem Tefera

Advisors: Ato Mistire Wolde, Dr. Daniel Seyfu, Dr, Dr.Frank Ashall andTedla Kebede.

Name of the Sponsor: Addis Ababa University College of Health Science, Department of Clinical Laboratory Science.

Aim of the study

This information sheet is prepared by a group of researchers at AAU for a project with the aim of compare insulin resistance and dyslipidemia in type 2 diabetes mellitus between groups of participant classified as hyperglycemic and normoglycemic participants.

Study design and procedure

If you agree to take part in the study, one of the investigators or a health worker will give you verbal and/or written information about the study and you will be given the consent from to sign, the physician or health professional will ask you some questions about your general health and perform a complete medical examination and assess whether you qualify to participate in the study. If you are fit for the study 10 ml of blood samples will also be collected for laboratory examination of insulin, blood glucose, HDL, LDL, total cholesterol, triglycerides, ApoA-I, and ApoB.

Risk and discomfort

Participating in this project will not cause more discomfort than is required you could go through for routine examination. But there could be minor pain and change in color of your skin following the blood drawing and which would disappear in short duration. If you have any discomfort, you can contact any of the investigators in this project. The amount of blood taken from each volunteer throughout the study period is 10-15ml which will not affect your health. There is no major risk in participating this research, as the whole procedure is carried out by physician and/or health professionals following the standard good clinical practice. Benefits and incentives.The result of the laboratory finding will be communicated to your physician for use in the management of the disease. You will have the chance to know your general health status from the medical examinations. And if the medical examination reveals any abnormalities that need immediate treatment, your doctor will be notified about the result. You will not be provided with any direct incentives for your participation in the research. But the cost for general medical examination will be covered by the project.

Confidentiality

All information about the patients will be kept confidential. Log books used in the laboratory will have no names but codes. The information sheet that links the coded number to patient name will be locked inside a box and it will not be revealed to anyone except your physician and the principal investigator. Right to refused or withdraw. You have full right to withdraw from participating in this study at any time before and after consent without explaining the reason. Your decision will not affect your right to get health service you are supposed to get otherwise.

Information sheet (Amharic version)

አዲስአበባዩኒቨርስቲየጤናሳይንስኮሌጅላብራቶሪትምሕርትክፍል

ጥናቱንስፖንሰርያደረገዉተቋምአዲስአበባዩኒቨርስቲጤናሣይንስኮሌጅ

የዚህጥናትአላማዉየስኳርሕመምበደማቸዉዉስጥበሚገኝግለሰቦችላይየኢንሱሊንሆርሞንመብዛትእናየደምቅባትመጨመርየሚ ባለዉንበሽታመፍጠርበሚያጠናዉጥናትላይለመሳተፍፈቃደኛለሆኑትየተዘጋጀየፈቃደኝነትመረጃየጥናቱንአላማናጥቅምየሚገል ጽበመጠየቁላይተሳታፊዎቸበጥናቱዉስጥላለመሳተፍእምቢማለትመብታቸዉእንደሆነናበማንኛዉምጊዜከጥናቱበራሳቸዉዉሳኔ መዉጣትጭምርመብታቸዉነዉ፡፡ከጥናቱበመዉጣታቸዉምምንምአይነትመንገላታትአይደርስባቸዉም፡፡

ስለሆነምሁኔታዉንበሚገባበማጤንፈቃደኛየሆኑትንተሳታፊዎችበምርምሩላይለመሳተፍይችላሉ፡፡በተጨማሪምተሳታፊዎቹየሚ ሰጡትየደምናሙናለCholesterol,Triglycerides,HDL-C LDL-C Glucose, HCV, FFAs እና Insulin ምርመራዎችብቻየሚዉልነዉ፡፡የጥናቱተሳታፊዎችማንኛዉንምያልገባቸዉንነገርየመጠየቅእድልአላቸዉበሚገባቸዉምቋንቋመ ልስያገኛሉ፡፡

በተጨማሪምየሁሉንምየላብራቶሪምርመራዉጤቶችበጊዜዉለሀኪሙይሰጥላቸዋልእናዉጤቱንመስማትከፈለጉበቀላሉማወቅና መረዳትይችላሉ፡፡

የፈቃደኝነትመግለጫፎርም

የሚስጥርቁጥር _____ የጥናቱተሳታፊፊርማ _____

የጥናቱተሳታፊስም _____

እኔስሜከላይየተገለጸዉግለሰብየተፈለኩትየስካርሕመምበደማቸዉዉስጥበሚገኝግለሰቦችላይየኢንሱሊንሆርሞንመብዛትእናየደ ምቅባትመጨመርየሚባለዉንበሽታመፍጠርበሚያጠናዉጥናትላይለመሳተፍመሆኑንናየጥናቱአላማናጥቅምተገልፆልኛል፡፡በመ ጠይቁላይየምሰጠዉየእኔሙሉመረጃእንደሚያዝተነግሮኛልበተጨማሪምጥናቱዉስጥላለመሳተፍእምቢማለትመብቴእንደሆነና በማንኛዉምጊዜከጥናቱበራሴዉሳኔመዉጣትጭምርመብትመሆኑንከጥናቱበመዉጣቴምንምአይነትመንገላታትእንደማይደርስ ብኝበሚገባተገልጾልኛል፡፡

ስለሆነምሁኔታዉንበሚገባበማጤንበፈቃደኝነትበምርምሩላይለመሳተፍለተመራማሪዉፈቃደኝነቴንሰጥቻለሁ፡፡በተጨማሪምየ ምሰጠዉየደምናሙናለCholesterol,Triglycerides,HDL-C LDL-C Glucose, FFAs እና Insulin ምርመራዎችብቻእንደሚዉልተነግሮኝተስማምቻለሁ፡፡ማንኛዉንምያልገባኝንነገርየመጠየቅእድልተሰጥቶኝበሚገባኝቋንቋመል ስእግኝቻለሁ፡፡

በተጨማሪምየሁሉምየላብራቶሪምርመራዉጤቶችበጊዜዉለሀኪሜእንደሚሰጥልኝእናዉጤቱንማወቅከፈለኩማግኘትእንደም ችልተነግሮኛል፡፡

እኔ _____

የተባልኩትግለሰብይህንሁሉበማገናዘብበምርምሩላይስለኔመረጃለመስጠትእናየደምናሙናለመስጠትተስማምቻለሁ፡፡

ፊርማ ቀን

ተሳታፊ _____ _____

ነርስ _____ _____

መጠይቅ
የጥናቱተሳታፊመለያከላይየተጠቀሱትስዱስትየወደፊዉጤታቸዉንለማወቅይጠቅማቸዋል

ክፍል 1 የመጠይቅመለያ

2. ጾታወንድሴት

3. የክብደትና

ክፍል 2-የሰዉነትልኬት

3. አሁንያለዉክብደት Kg 4. -አሁንያለዉቁመት CM

5. የክብደትናየቁመትልዩለት KG/m2 6. -የዳሌዙሪያልኬት CM

7. የወገብዙሪያ CM 8. የወገብናየዳሌዙሪያልዩነት

9. የደምግፊትሲስቶሊክ 10. የደምግፊትዳያስቶሊክ mm/Hg

10 የሰዉነትሙቀት 0^{C}

ክፍል 3 የአመጋገብመረጃ .

2 የሚከተሉትንምግቦችምንያህልጊዜበተደጋጋሚትመገባላችሁ

1=በፍጹም 2= አልፎአልፎ (በበአል፣በሰርግ፣በክብረበአል፣ወ.ዘ.ተ) 3=አንዴወይምሁለቴ /በወር

4= 1-4/በሳምንት 5=አንዴወይምሁለቴ/ በቀን

1. ወተትናየወተትዉጤቶች(አይብ፣ቅቤ፣ጮማ)

2. ስጋ (ዶሮን፣አሳወዘተ)

3. እንቁላል

4. ሀይልሰጪዎች(ዳቦ፣ፓስታወዘተ)

2.መጠጥይጠጣሉወይአልጠጣምአዎ

አዎካሉምንያህልጊዜይጠጣሉ ? በየቀኑ በየሳምንቱ አልፎአልፎ

3.ያጨሳሉ? አዎአላጨስም

አዎካሉምንአይነትአጫሽኖት ? ሁልግዜየሚያጨስአልፎአልፎየሚያጨስ

4.እንቅስቃሴያደርጋሉ? አዎ አላደርግም

አዎካሉምንያህልጊዜ? በየቀኑ በሳምንትከሁለትእስከሶስትቀን አልፎአልፎ