Faculty of Medicine

Thesis title:

Lipoprotein (a) as atherogenic risk factor in Libyan type II diabetic patients

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DEDICATION

TO MY

DEAR FAMILY

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ABBREVIATIONS

ABC	ATP-Binding Cassette transporter
ADA	American Diabetes Association
AGE	Advanced Glycation End-products
ANOVA	Analysis of Variance
AT1	Angiotensin II receptor type 1
BMI	Body Mass Index
BP	Blood Pressure
CAD	Coronary Artery Disease
cAMP	Cyclic Adenosine Monophosphate
CD	Cluster Determinant
CE	Cholesterol Ester
CETP	Cholesterol Ester Transfer Protein
CVD	Cardiovascular Disease
DBP	Diastolic Blood Pressure
DM	Diabetes Mellitus
EC	Endothelial Cell
ECL	Electrochemiluminescence
EDTA	Ethylenediaminetetraacetic acid
eNOS	endothelial Nitric Oxide Synthase
FBS	Fasting Blood Sugar
FC	Free Cholesterol
FFA	Free Fatty Acid
FGF	Fibroblast Growth Factor
FXR	Farnesoid X Receptor
G6PDH	Glucose-6-Phosphate Dehydrogenase
GDM	Gestational Diabetes Mellitus
GIP	Gastric inhibitory Polypeptide
GK	Glycerol Kinase
GLP	Glucagon Like Peptide
GPO	Glycerophosphate Oxidase
HbA1c	Glycosylated Hemoglobin
HDL	High Density Lipoprotein
HK	Hexokinase
HNF	Hepatocyte Nuclear Factor

HSDA	Hydroxy Sulfopropyl Dimethoxyaniline
HSL	Hormone Sensitive Lipase
HSPG	Heparin Sulfate Proteoglycan
IDF	International Diabetes Federation
IRS	Insulin Receptor substrate
K	Kringle
LDL	Low Density Lipoprotein
Lp(a)	Lipoprotein (a)
LPL	Lipoprotein Lipase
LRP	LDL Receptor-related Protein
M-CSF	Macrophage Colony-Stimulating Factor
MENA	Middle East and North Africa
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NO	Nitric Oxide
ox-Lp(a)	oxidized Lp(a)
PAI	Plasminogen Activator Inhibitor
PEG	Polyethylene Glycerol
PLTP	Phospholipid Transfer Protein
POD	Peroxidase
RAS-ERK	RAS-Extracellular signal-Regulated Kinase
SBP	Systolic Blood Pressure
SD	Standard Deviation
sdLDL	small dense Low Density Lipoprotein
SMC	Smooth Muscle Cell
SP conjugate	Streptavidin Peroxidase Conjugate
sPLA2	secretory Phospholipase A2
SPSS	Statistical package for the social sciences
SR-B	Class B Scavenger Receptor
T2DM	Type 2 Diabetes Mellitus
TAG	Triacylglycerol
TC	Total Cholesterol
TGF-β	Transforming Growth Factor-β
TMB	Tetramethylbenzidine
TNF	Tumor Necrosis Factor
t-PA	tissue Plasminogen Activator
UCP	Uncoupling Protein

VCAM-1	Vascular Cell Adhesion Molecule 1
VLDL	Very Low Density Lipoprotein
WHO	World Health organization

ABSTRACT

Type 2 diabetic patients have increased morbidity and mortality due to cardiovascular risk events. This increased risk has been shown to be independent from conventional risk factors. Different factors have been found to be responsible for an increased prevalence of CAD in T2DM. One of these are the raised serum levels of lipoprotein (a). The present case-control study designed to evaluate the association of the Lp(a) levels with T2DM in Libyans, in addition to its association with the degree of glycemic control, insulin, and lipid profile.

A total of 100 type 2 diabetic patients were recruited from the Benghazi Center for Diagnosis and Treatment of Diabetes, and Alhaia Clinic in Benghazi, and 30 apparently healthy age and sex- matched individuals were selected from the High Institute of Medical Professions-Benghazi, to serve as controls. Clinical information and medical history were obtained through a questionnaire from all diabetic and normal control subjects. Blood samples were collected and analyzed for Lp(a), FBS, HbA1c, insulin, TC, TAG, LDL-c, and HDL-c.

Lipoprotein (a) was significantly higher in diabetic patients when compared to control subjects. Lp(a) was positively correlated with TC, and LDL-c. No significant correlations were found between Lp(a) and glycemic control parameters, insulin, TAG, HDL-c, BMI, and BP.

These results are in line with other studies that reported that CVD risk in type 2 diabetic patients may be dependent on additional lipid risk factors and indicated that LDL-c may not be an independent risk factor for the development and progression of atherogenesis in type 2 DM. Lp(a) may to be a new member of metabolic syndrome and it may be useful in routine clinical practice in future as a cardiovascular risk marker.

Chapter 1

Review of Literature

1. Review of literature

1.1. Diabetes Mellitus:

Diabetes mellitus is a metabolic disorder of multiple etiologies. It is characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects of insulin secretion, insulin action or both (Alberti and Zimmet, 1998).

1.1.1. Classification:

Diabetes mellitus is classified by the World Health Organization (WHO) according to etiology to:

1- Type 1 diabetes:

This form of diabetes accounts for only 5–10% of those with diabetes, and results from cellular-mediated autoimmune destruction of the pancreatic β-cells. T1DM was previously known as insulin dependent or juvenile-onset diabetes (American Diabetes Association, 2012).

2- Type 2 diabetes:

T2D accounts for 90–95% of those with diabetes, previously encompassed by the terms non–insulin-dependent diabetes, or adult onset diabetes. Its main feature is insulin resistance accompanied by insulin deficiency (i.e. patients secrete insulin, but not enough to overcome the insulin resistance) (Holt et al., 2011).

3- Gestational Diabetes Mellitus (GDM):

Gestational diabetes mellitus is a circumstantial classification (rather than a pathophysiologic condition) identifying women who develop diabetes mellitus during gestation (Reasner and Defronzo, 2006).

4- Other Specific Types:

The various types of diabetes with known etiologies are grouped together to form the classification called "Other Specific Types". This group includes patients with genetic

defects of beta-cells or their action; patients with diseases of the exocrine pancreas, such as pancreatitis; patients with dysfunction associated with other endocrinopathies (e.g. Acromegaly); and patients with pancreatic dysfunction caused by drugs, chemicals or infections (Patidar and Dwivedi, 2012).

1.2. Type 2 diabetes mellitus:

1.2.1. Epidemiology:

Over the past three decades, the number of people with type II diabetes mellitus has more than doubled globally, making it one of the most important public health challenges to all nations. The increasing prevalence of type II diabetes is a result of population ageing, urbanization and associated life style changes (Zimmet et al., 2001). The total number of people worldwide with type II diabetes was expected to increase from 171 million in 2000 to 366 million in 2030 (Wild et al., 2004). Unfortunately, the prevalence worldwide already reached 366 million by 2011 according to the International Diabetes Federation (IDF), which estimated that the figure is expected to reach 439 million by 2030 (Whiting et al., 2011), and the disease accounts for 4.6 million deaths in 2011 for people aged 20 – 79 years, accounting for 8.2% of global all-cause mortality for people in this age group with a rate of one death every seven seconds (Federation, 2011), while In 2013, IDF announced that 382 million people worldwide or 8.2% of adults have type 2 diabetes, and by 2035 this figure will rise to 592 million. The federation also estimated that about 80% of patients live in low- and middle- income countries (Guariguata et al., 2014), and the disease is responsible for 5.1 million deaths in 2013, or one death every six seconds.

In Africa, nearly 19.8 million people or 4.9% of the adult population are affected. By 2035 this number is expected to rise to 41.5 million. The highest prevalence of diabetes in the Africa Region is on the island of Reunion (15.4%), followed by Seychelles (12.1%), Gabon (10.7%) and Zimbabwe (9.7%). Of all mortalities in 2013, diabetes caused about 8.6% (Federation, 2013).

In Middle East and North Africa region, about 9.2% of adult population, equivalent to 34.6 million people has diabetes. The figure is expected to increase to 67.9 million by 2035. The Gulf region has the highest prevalence of diabetes, with Saudi Arabia, Kuwait, Qatar and Bahrain has prevalence of 24%, 23.1%, 22.9%, and 21.8% respectively, and is of the world's highest prevalence. United Arab Emirates has a prevalence of about 19%. Prevalence of diabetes is also very high in countries that are not of the Gulf region, such as Egypt (16.8%), Lebanon (14.9%), and Oman (14.2%). Prevalence in Tunisia, Algeria, and Sudan are 9.5%, 7.4%, and 9.4% respectively (Majeed et al., 2014). Diabetes was responsible for about 10% of all deaths in MENA region in 2013 (Federation, 2013).

In 1999, a study of Kadiki et al showed that the prevalence of T2DM in Benghazi was about 19% of the adult population, and the most common complication of diabetes was neuropathies (45.7% of all complications) (Kadiki and Roaed, 1999). In 2011, IDF announced that the comparative prevalence of diabetes in Libya was about 14.1% (Federation, 2011), then in 2013 they estimated the comparative prevalence of diabetes at about 9.8%, and the national prevalence at about 8.43% of the adult population (Federation, 2013).

In a study of 952 type 2 diabetic patients in Benghazi, about 68.7% had long term complications of diabetes. 14.9% of patients had coronary heart disease, 30.6% had diabetic retinopathy, 47.1% had peripheral neuropathy, 25.8% had macroalbuminuria, 15.2% had peripheral arterial disease, and 13.1% had cataract (Roaeid and Kadiki, 2011). In Tripoli, diabetes was responsible for about 26.2% of all mortalities during a period of three years (2005 – 2008)(Abduelkarem et al., 2010), whereas in Benghazi, diabetes accounted for 12.1% of all deaths during a period from 2000 to 2004 (Roaeid and Kablan, 2010).

1.2.2. Diagnosis:

The diagnosis of T2D, as currently outlined by the American Diabetes Association (ADA), is based on an A1c $\geq$ 6.5%, or fasting plasma glucose level $\geq$ 126 mg/dL,

or 2-h plasma glucose ≥ 200 mg/dL during an oral glucose tolerance test, or the presence of classical symptoms of hyperglycemia (polyuria, nocturia, polydypsia, etc) and a random plasma glucose ≥ 200 mg/dL (American Diabetes Association, 2011).

1.2.3. Etiology:

Type 2 diabetes mellitus (T2DM) is a heterogeneous disorder caused by a combination of genetic and environmental factors which adversely affect β - cell function and tissue insulin sensitivity (Gerich, 1998) .

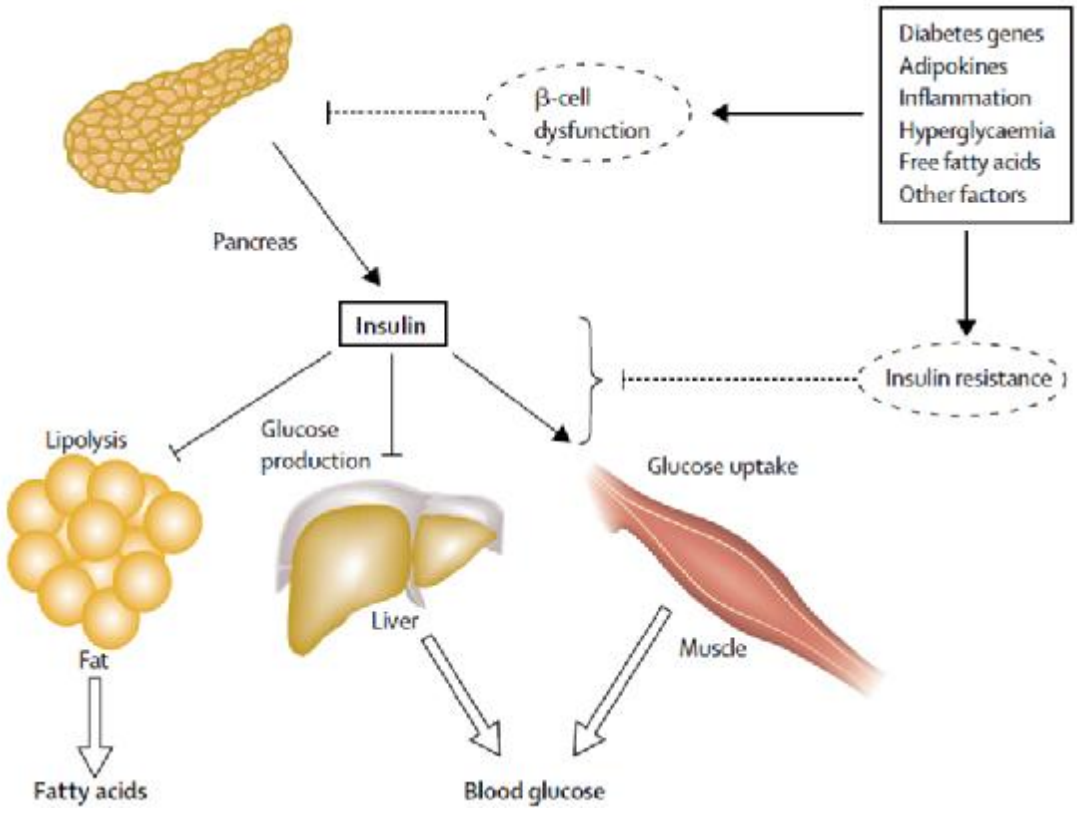
Insulin is the key hormone for regulation of blood glucose. In skeletal muscle, insulin stimulates glucose transport, glycolysis, glycogenesis, and tricarboxylic acid cycle activity. In the liver, Insulin inhibits glucose output by down regulating glycogenolysis and gluconeogenesis, and stimulates glycogenesis. In adipose tissue, insulin promotes glucose transport, glycerol formation, and triacylglycerol synthesis, while at the same time exerting an antilipolytic effect. During periods of fasting, the circulating levels of insulin falls and that of counter regulatory hormones increases leading to breakdown of stored fuels and increased availability of metabolic substrates for cellular energy.

Insulin resistance is the failure of insulin to produce its usual biologic effects at circulating concentrations that are sufficient in normal subjects.

Insulin resistance leads to impaired suppression of hepatic glucose production and to reduced peripheral uptake of glucose. Resistance to the ability of insulin to suppress very low density lipoprotein (VLDL) cholesterol production leads to increased serum triacylglycerols, while resistance in adipose tissue impairs the ability of insulin to inhibit lipolysis, and results in increased circulating free fatty acids (Holt et al., 2011). Insulin resistance is largely associated with obesity and physical inactivity. Furthermore, insulin action is modulated by a number of circulating hormones, cytokines, and metabolic fuels, such as free fatty acids, that are originated in the adipocytes. An increased mass of stored triacylglycerol, especially in visceral or deep subcutaneous adipose tissue, leads to large adipocytes that are themselves resistant to the insulin action on lipolysis. This leads to increased release and circulating levels of

FFA and glycerol, both of which exacerbate insulin resistance in skeletal muscle and liver (Boden, 1997) (Figure 1.1).

The genetic factors implicated in insulin resistance, include not only insulin receptor and insulin receptor substrate (IRS)-1 gene polymorphisms that directly affect insulin



Stumvoll, M., Goldstein, B. J. & Van Haeften, T. W. 2005. Type 2 diabetes: principles of pathogenesis and therapy. The Lancet, 365**,** 1333-1346.

Figure 1.1: Pathophysiology of hyperglycaemia and increased circulating fatty acids in type 2 diabetes.

signals but also polymorphisms of thrifty genes such as the β_3 adrenergic receptor gene and the uncoupling protein (UCP) gene, associated with visceral obesity and promote insulin resistance (Kohei, 2010).

Initially, insulin secretion from pancreatic β cells increases in response to insulin resistance to maintain normal glucose levels (Stumvoll et al., 2005). T2DM develops

because of a progressive deterioration in β-cell function coupled with the addition of acquired insulin resistance for which the β-cell cannot compensate (Kahn, 2001). At time of diagnosis, β-cell function is already reduced about 50% (Group, 1995).

In T2DM, the impairment of β-cell function results from a combination of genetic and acquired factors. T2DM is a polygenic disorder in which multiple genes (i.e. polymorphisms), each insufficient in themselves, must be present with or without acquired abnormalities in order to cause diabetes (Bell and Polonsky, 2001). Such genes may cause defects in β-cell regeneration, apoptosis, glucose sensing, glucose metabolism, energy transduction, ion channels, microtubules and other islet proteins necessary for the synthesis, packaging, movement and release of secretory granules (Stumvoll et al., 2007).

Acquired factors include glucotoxicity, lipotoxicity, obesity, alterations in incretins (GLP-1, GIP), interleukin 1β which induces β cells apoptosis, β cells cytotoxic islet amyloid, and malnutrition in utero and in early life which may affect programing of the β-cell with respect to glucose sensing, apoptosis, regeneration and the ability to compensate for insulin resistance (Holt et al., 2011).

1.3. Plasma lipoproteins:

1.3.1. Chylomicrons:

Chylomicrons are mainly synthesized in the jejunum after a meal, during the postprandial phase. They are lowest density lipoprotein particles and are very heterogeneous in size (Vors et al., 2013). Chylomicron particle consists of a core of neutral lipids, predominantly TAG, with traces of cholesteryl ester, surrounded by a shell of amphipathic lipids (phospholipids, cholesterol) and one structural protein, the apolipoprotein (apo) B48, as well as other exchangeable apolipoproteins (Vance and Vance, 2008).

In the blood circulation, chylomicrons' triacylglycerols are hydrolyzed by lipoprotein lipase into free fatty acids that are taken up by organs such as the adipose tissue for storage, or skeletal muscles and the heart for energy supply. The chylomicron

remnants are endocytosed by the liver, especially by the LDL receptor that has a high affinity for apoE, but other routes are involved including the LDL receptor-related protein 1 (LRP1) and the heparin sulfate proteoglycan (HSPG) pathway (Hassing et al., 2012, Lambert and Parks, 2012).

1.3.2. Very low density lipoprotein:

VLDL is synthesized in the liver. It is composed of a large amount of de novo synthesized TAG, apo B-100, apoC, and apoE. In the blood circulation, it incorporates additional apoC from HDL particles (Crook, 2012). The fatty acids required for TAG synthesis are derived from de novo synthesis using glucose as substrate or from lipolysis in adipose tissue by the action of adipose tissue TAG lipase, and hormone sensitive lipase (Yen et al., 2008), which is inhibited by insulin (Evans et al., 2002). Whereas insulin inhibits lipolysis in adipose tissue during postprandial period, it activates the enzymes involved in fatty acid and TAG synthesis (lipogenesis) in adipose tissue and liver (Holt et al., 2011). VLDL is hydrolysed by lipoprotein lipase in peripheral tissues (Crook, 2012). The amount of free fatty acids released from chylomicrons and VLDL depends on the activity of LPL, which is stimulated by insulin (Karpe et al., 2011). The liberated FFAs are taken by adipose tissue for storage, and by heart and skeletal muscle for energy (Evans et al., 2002). The resulting VLDL remnant contains cholesterol and triacylglycerol as well as apoB and apoE and is rapidly taken up by the hepatic receptors or modified by the action of hepatic lipase to LDL by losing apoE and triacylglycerol (Crook, 2012). Many studies have suggested that VLDL synthesis is inhibited by insulin (Malmström et al., 1997).

1.3.3. Low density lipoprotein:

LDL is a small cholesterol rich lipoprotein particle containing only apoB100. It constitutes about 70% of the total plasma cholesterol concentration. It can be taken up by most cells, especially hepatocytes by the LDL or B/E receptor which recognizes and bind apoB100. Inside the cell, the LDL particles are broken down by lysosomal enzymes, releasing cholesterol. This cholesterol can be used to synthesize steroids in

specific tissues such as the adrenal cortex or gonads or incorporated into cell membranes. Most cells synthesize cholesterol, but there is a feedback control system reducing the rate of LDL receptor synthesis and de novo cholesterol synthesis by cholesterol itself, to avoid intracellular cholesterol accumulation.

If the plasma cholesterol concentration is excessive, LDL particles can infiltrate tissues by passive diffusion and can even cause damage, as in atherosclerosis. An alternative route of LDL removal from plasma is via the reticuloendothelial system, which recognizes only chemically modified LDL, for example oxidized LDL (Vance and Vance, 2008, Crook, 2012).

1.3.4. High density lipoprotein:

In human plasma, most of the HDLs are spherical particles that contain a hydrophobic core of cholesteryl esters and a small amount of triacylglycerol stabilized by a monolayer of phospholipids especially phosphatidylcholine, unesterified cholesterol, and apolipoproteins mainly apo A-I (Dixon and Ginsberg, 1992). HDLs are surrounded by the rest of apo A class, apo C, and apo E apolipoproteins (Murray et al., 2009). HDL can be classified on the basis of density, resulting in the large HDL2 and the small dense HDL3, which can be further subclassified into 5 distinct subpopulations (HDL2a, HDL2b, HDL3a, HDL3b and HDL3c) (Camont et al., 2011). Apo A-I is synthesized in liver and intestine and secreted into the plasma in lipid free or lipid poor form. After hepatic secretion, it acquires phospholipids and unesterified cholesterol from cell membranes and other lipoproteins in the blood circulation and is converted into discoidal HDL (pre-β-HDL). These nascent particles interact with peripheral tissues acquiring more FC, which is then converted to cholesteryl ester by lecithin-cholesterol acyl transferase (LCAT) (Pirillo et al., 2013), the enzyme that is activated by apo A-I (Panagotopulos et al., 2001). The hydrophobic cholesteryl esters (CE) move into the center of the particles, resulting in the formation of spherical lipoproteins HDL3, the major type of circulating HDL. Small HDL3 particles acquire more cholesterol, and are converted into large spherical HDL2 particles that contain

a lipid core composed of CE and triacylglycerol (Rye and Barter, 2004, Pirillo et al., 2013). Spherical HDL can be remodeled by lipases especially hepatic lipase in liver, which hydrolyses HDL phospholipids and triacylglycerols resulting in reduction in HDL size, formation of lipid-poor HDL particles and release of lipid-free apoA-I, which can restart the lipidation cycle (Clay et al., 1991, Pirillo et al., 2013). Cholesteryl ester transfer protein (CETP) and Phospholipid transfer protein (PLTP) contribute to the HDL remodeling. CETP remodels HDLs by transferring cholesteryl esters from HDLs to VLDLs in exchange for triacylglycerols (Barter et al., 2003). CETP can also mediate bi-directional equimolar exchanges of cholesteryl esters between HDLs and LDLs (Barter and Jones, 1979). PLTP transfers phospholipids between different HDL particles, as well as between HDL and other plasma lipoproteins (Rao et al., 1997).

A major function of HDL is to act as a reservoir for the apo C and apo E required in the metabolism of chylomicrons and VLDL. HDL promotes the uptake of cholesterol from peripheral tissues, including the arterial wall, and returns cholesterol to the liver for excretion via the bile (either as cholesterol or after conversion to bile acids), in a process known as reverse cholesterol transport.

Class B1 scavenger receptor (SR-B1) has been identified as an HDL receptor with a dual role in HDL metabolism. In the liver and in steroidogenic tissues, it binds HDL via apo A-I, and cholesteryl ester is selectively delivered to the cells. In the tissues, on the other hand, SR-B1 mediates the acceptance of cholesterol effluxed from the cells by HDL, which then transports it to the liver (Vance and Vance, 2008).

A second important mechanism for reverse cholesterol transport involves the ATP-binding cassette transporter A1 (ABCA1). ABCA1 is a member of a family of transporter proteins that couple the hydrolysis of ATP to the binding of a substrate, enabling it to be transported across the membrane. ABCA1 preferentially transfer cholesterol from cells to poorly lipidated particles such as pre-β-HDL or apo A-1, which are then converted to HDL3 (Remaley et al., 2001, Vance and Vance, 2008).

1.4. Diabetic dyslipidemia and Related CVD:

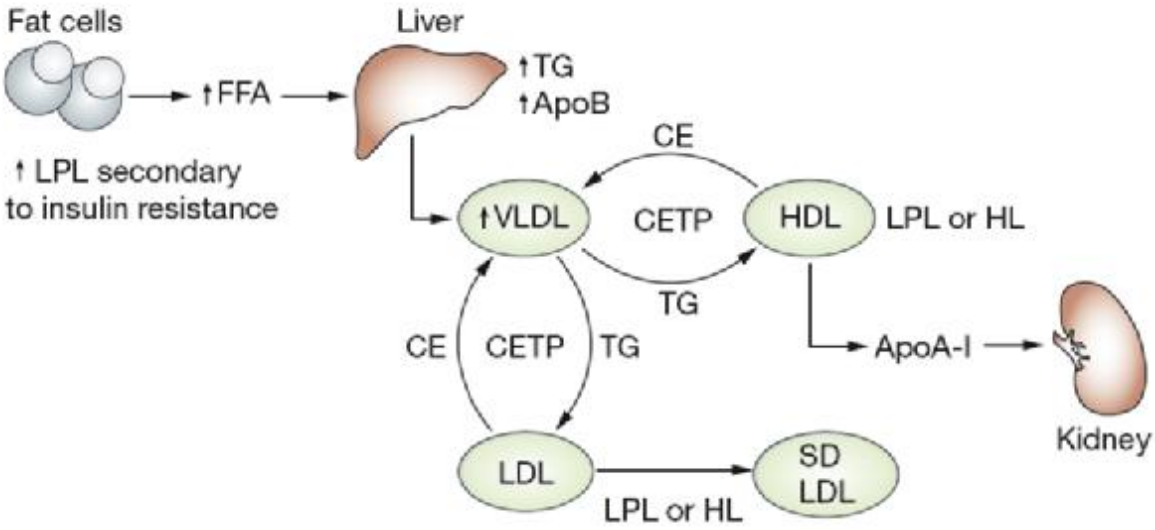
Many epidemiologic studies have demonstrated that diabetes mellitus is an independent risk factor for cardiovascular disease and that it aggravates the effects of other common risk factors, such as smoking, hypertension, and hypercholesterolemia (Stamler et al., 1993, Almdal et al., 2004). The mortality associated with cardiovascular diseases in people with diabetes mellitus is significantly higher than the mortality in nondiabetic individuals (Sprafka et al., 1991). The increased risk of atherosclerosis in diabetics consists of multiple factors. Diabetes-related changes in plasma lipid levels, which are collectively known as diabetic dyslipidemia or atherogenic dyslipidemia, are among the key factors that increase the risk of CVD in diabetics (Hachem and Mooradian, 2006).

Diabetic dyslipidemia is attributed mostly to insulin resistance and insulin deficiency and characterized by high plasma triacylglycerol concentration, low HDL cholesterol concentration and increased concentration of small dense LDL–cholesterol particles (Taskinen, 2003, Chahil and Ginsberg, 2006).

Type 2 diabetic patients exhibit a lack of insulin action on hepatic lipid metabolism, which normally limits the synthesis of VLDL1, and on the lipolytic activity of lipoprotein lipase. Impaired insulin action also results in defective suppression of lipolysis in adipose tissue with the influx of the released free fatty acids to the liver.

This scenario induces a dramatic increase of VLDL1 production, the largest lipoproteins rich in triacylglycerols (Adiels et al., 2008, Krentz, 2003). The increased concentration of VLDL1 in type 2 diabetic patients is mainly observed in the postprandial period. This pattern of metabolic impairment is maintained for many hours upon food intake, exposing the patient to a prolonged period of hypertriacylglycerolemia and hyperchilomicronemia (Ginsberg and Illingworth, 2001, Panarotto et al., 2002). Under hypertriacylglycerolemic conditions, there is excessive exchange of cholesterol esters and triacylglycerols between HDL and VLDL, mediated by cholesterol ester transfer protein (CETP). Hepatic lipase hydrolyzes TAG-rich HDL particles and, as a result, are rapidly catabolized and cleared from

plasma. Reduced activity of LPL may further decrease levels of HDL cholesterol by decreasing the conversion of HDL3 to HDL2 particles (Mooradian et al., 2008). Increased concentrations of VLDL particles in the circulation of patients with T2DM also increase the CETP-mediated exchange of cholesterol ester and triacylglycerol between VLDL and low density lipoprotein (LDL) cholesterol particles. This increase in triacylglycerol content of LDL particles makes them a good substrate for hepatic lipase, which hydrolyzes triacylglycerols in the LDL particles and increases their density, resulting in increased concentration of small dense LDL particles (Krauss and Ronald, 1998, Krauss, 1998) (Figure 1.2).



Mooradian, A. D. 2009. Dyslipidemia in type 2 diabetes mellitus. Nature clinical practice endocrinology & metabolism, 5**,** 150-159.

Figure 1.2: The role of insulin resistance in diabetic dyslipidemia.

Hypertriacylglycerolemia, in conjunction with excessive small dense LDL cholesterol concentration and low HDL cholesterol concentration, is an important contributor to accelerated atherosclerosis in type 2 diabetic patients (Detection, 2001). Many studies

have shown that LDL cholesterol is the strongest risk factor for CVD, followed by low HDL as the second strongest, then triacylglycerol (Turner et al., 1998b).

Small dense LDL has a reduced binding affinity to the LDL receptor and increased binding affinity to arterial wall proteoglycans. The result is greater existence of small dense LDLs in blood circulation, which easily adhere to the endothelial surface and are more easily captured in the subendothelium space (Skålén et al., 2002, Anber et al., 1996). The prolonged presence of sdLDL particles in the circulation enables their glycation and oxidation (Soran and Durrington, 2011). Thus, they can be taken up by the scavenger receptor of the macrophage which has a much greater affinity for modified LDL than non-modified. The reduction in HDL-c level is accompanied by a reduction of the HDLs' antiatherogenic properties, which in addition to reverse cholesterol transport from the arterial wall to the liver (Tan, 2009) include :

1. Anti-oxidative properties due to glutathione, paraoxonase, and selenoperoxidase enzymes.
2. Anti-inflammatory properties by suppression of endothelial tumor necrosis factor alpha (TNF-α) production, cytokine-mediated expression adhesion molecules, and serum amyloid A protein.
3. Anti-thrombotic properties by reduction of platelet aggregation by platelet activating factor and thromboxane.
4. Vasodilatory action by increasing the activity of endothelial NO synthase (eNOS) (Leeper and Heidenreich, 2008, Rader, 2012).

1.4.1. Cardiovascular disease:

Cardiovascular disease is a broad term for a number of pathological conditions of blood vessels associated mainly with the heart and brain. The major causes of death are coronary heart disease and stroke. The common, chronic condition behind these diseases is atherosclerosis. Atherosclerosis can result in complete blockage of coronary artery, resulting in coronary heart disease or stroke if it is in the brain (Gurr et al., 2002).

Atherosclerosis is a disease of large- and medium-sized arteries that is characterized by a formation of atherosclerotic plaques consisting of necrotic cores, calcified regions, accumulated modified lipids, endothelial cells (ECs), inflamed smooth muscle cells (SMCs), leukocytes, and foam cells (Epstein and Ross, 1999).

A primary initiating event in atherosclerosis is LDL retention in the subendothelial matrix. LDL accumulation is greater when their circulating levels are raised. LDL diffuses passively through EC junctions, and its retention in the vessel wall seems to involve interactions between the LDL constituent apolipoprotein B and matrix proteoglycans (Borén et al., 1998). Trapped LDL particles undergo modification, including lipolysis, oxidation, proteolysis and aggregation, and such modifications contribute to inflammation and foam-cell formation. One of the modifications most significant for early lesion formation is lipid oxidation as a result of exposure to the oxidative waste of vascular cells. Such modifications initially give rise to 'minimally oxidized' LDL species that have pro-inflammatory activity but may not be sufficiently modified to be recognized by macrophage scavenger receptors (Lusis, 2000).

Small dense LDL has a decreased binding affinity to the LDL receptor and increased binding affinity to arterial wall proteoglycans. The result is a greater persistence of circulating small dense cholesterol-rich LDL lipoproteins which easily adhere to the endothelial surface and are more easily captured in the subendothelium space (Anber et al., 1996, Skålén et al., 2002). The prolonged presence of sdLDL particles in blood enables their modification more than native LDLs (Soran and Durrington, 2011). High-density lipoprotein (HDL) is strongly protective against atherosclerosis. An important mechanism contributing to this protective effect is the role of HDL in the removal of excess cholesterol from peripheral tissues. But in addition, HDL also protects by inhibiting lipoprotein oxidation. The antioxidant properties of HDL are due in part to its serum paraoxonase content, an esterase carried on HDL that can degrade certain biologically active oxidized phospholipids (Hegele, 1999, Shih et al., 2000). HDL have other antiatherogenic properties including Anti-inflammatory properties by suppression of endothelial tumor necrosis factor alpha (TNF-α)

production, cytokine-mediated expression adhesion molecules, and serum amyloid A protein, Anti-thrombotic properties by reduction of platelet aggregation by platelet activating factor and thromboxane, and Vasodilatory action by increasing the activity of endothelial NO synthase (eNOS). Therefore, the reduction in HDL-c level is accompanied by a reduction of the HDLs' antiatherogenic properties (Leeper and Heidenreich, 2008, Rader, 2012).

Atherosclerosis involves the recruitment of lymphocytes, and monocytes to the artery wall. A triggering event for this process is the accumulation of minimally oxidized LDL, which stimulates the overlying ECs to produce a number of proinflammatory molecules, including adhesion molecules and growth factors such as macrophage colony-stimulating factor (M-CSF) (Watson et al., 1997). Oxidized LDL can also block the production of nitric oxide (NO), a chemical mediator with multiple anti-atherogenic properties, including vasorelaxation (Knowles et al., 2000). In addition to oxidized LDL, many other factors are likely to modulate inflammation, including homocysteine levels, haemodynamic forces, sex hormones, and infection. Diabetes may promote inflammation in part by the formation of advanced end products due to glycation that interact with endothelial receptors (Hofmann et al., 1999, Lusis, 2000). The entry of specific leukocytes into the artery wall is mediated by adhesion molecules and chemotactic factors. The first step in adhesion, the 'rolling' of leukocytes along the endothelial surface is mediated by selectins, which bind to carbohydrate ligands on leukocytes (Dong et al., 1998, Collins et al., 2000). The cytokine M-CSF induces the proliferation and differentiation of macrophages, and modulates various macrophage functions such as expression of scavenger receptors (Smith et al., 1995).

LDL must be extensively modified (highly oxidized) before it can be picked up sufficiently rapidly by macrophages to form foam cells. This modification involves reactive oxygen species produced by ECs and macrophages, but several enzymes are also involved, including myeloperoxidase, sphingomyelinase and a secretory phospholipase, all of which occur in human atherosclerotic lesions. Myeloperoxidase

generates highly reactive species such as hypochlorous acid and tyrosyl radical, and myeloperoxidase-modified LDL binds to macrophage scavenger receptors (Podrez et al., 2000). Sphingomyelinase may promote lipoprotein aggregation, resulting in increased retention and enhanced uptake by macrophages (Marathe et al., 1999). Finally, a secretory phospholipase (group II sPLA2) can promote LDL oxidation (Ivandic et al., 1999). The rapid uptake of highly modified LDL particles by macrophages, leading to foam-cell formation, is mediated by a group of receptors that recognize a wide array of ligands such as scavenger receptors (SR-A and CD36) (Suzuki et al., 1997, Febbraio et al., 2000). Lipid uptake by scavenger receptor is not controlled by feedback regulation (Gurr et al., 2002).

Macrophages express CD40 receptor and the engagement of this receptor and CD40 ligand expressed on T cells results in the secretion of cytokines, and growth factors, that are important for SMC migration and proliferation and extracellular matrix production to form fibrous plaques which are characterized by a growing mass of extracellular lipid, mostly cholesterol and its ester, and by the accumulation of SMCs and SMC-derived extracellular matrix (Schönbeck et al., 2000b).

Advanced atherosclerotic lesions lead to ischemic symptoms as a result of progressive narrowing of the vessel lumen. Myocardial infarction and stroke are generally thought to result from plaque rupture and thrombosis (Lee and Libby, 1997). Vulnerable plaques generally have thin fibrous caps and increased numbers of inflammatory cells. Rupture frequently occurs at the lesion edges, which are rich in foam cells (Libby, 2000), and exposes plaque lipids and tissue factor to blood components, initiating the coagulation cascade, platelet adherence, and thrombosis (Schönbeck et al., 2000a).

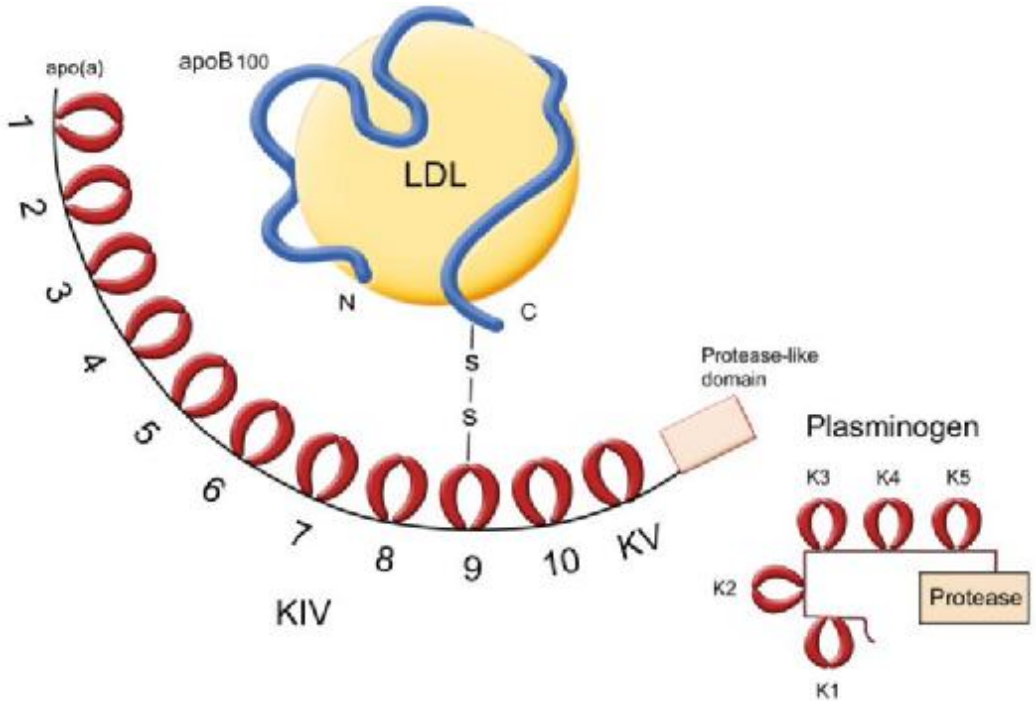
1.5. Lipoprotein (a):

Lp (a) has been described nearly 50 years ago by Kare Berg (Berg, 1963), and has been considered a genetic variant of low density lipoprotein. After many years, it has been recognized as a distinct class of lipoproteins (Dubé et al., 2012). At present, the true physiologic role of apoA has not been detected (Yano et al., 1997), and many studies have shown that elevated plasma Lp (a) levels contribute significantly to the incidence of cardiovascular diseases (Marcovina et al., 2003).

1.5.1. Structure:

Lipoprotein (a) consists of an LDL-like core lipoprotein and glycoprotein apo(a) covalently linked by a disulfide bond. The disulfide bridge links Cys4326 in apoB-100 with the only free Cys4057 in apo(a), located in kringle four (K-IV) type 9. The lipid core of Lp(a) is very similar to that of LDL. Apo(a), the characteristic glycoprotein component of Lp(a) consists of repetitive protein segments, so-called kringles (K) that are highly homologous to K-IV of plasminogen (McLean et al., 1987). The N-terminal part of apo(a) consists of multiple repetitive copies of these kringle-IVs. Apo(a) in addition has one copy of a K-V like and a protease-like domain similar to plasminogen. The protease domain of apo(a) lacks enzymatic activity (Figure 1.3).

In humans, 30 or more genetically determined apo(a) isoforms exist, giving rise to substantial size heterogeneity. The smallest apo(a) isoform consists of the protease domain, one copy of K-V and 11 K-IVs of which K-IV type 1 and K-IV types 3 to 10 occur once, whereas K-IV type 2 is present in two identical copies. Larger apo (a) isoforms differ by the number of K-IV type 2; the largest apo(a) has 52–54 K-IVs. The K-IVs are connected by linkers that are highly glycosylated with N- and O-linked sugars. Although apo(a) is mostly associated with LDL, there are small and variable amounts of free apo(a) present in the plasma (Gries et al., 1987).



Hoover-Plow, J. & Huang, M. 2013. Lipoprotein (a) metabolism: potential sites for therapeutic targets. Metabolism, 62**,** 479-491.

Figure 1.3: Lipoprotein (a) structure.

1.5.2. Metabolism:

Apo(a) is synthesized almost exclusively in the liver and follows classical steps of glycoprotein biosynthesis. Plasma Lp(a) levels are highly correlated with apo(a) production (Kostner et al., 2013). The transcription of genes involved in lipid and lipoprotein metabolism is strongly regulated by nuclear receptors including peroxisome proliferator-activated receptors, hepatocyte nuclear factors (HNFs), retinoid X receptor, liver X receptor, and farnesoid X receptor (FXR) (Chawla et al., 2001). FXR activation by bile acids reduce plasma concentrations and hepatic expression of human LPA (Chennamsetty et al., 2011). Bile acids have a dual influence on apo(a) expression. Apo(a) expression is driven by the specific liver transcription factor HNF4α. The apo(a) promoter has several putative-binding sites for HNFs. In the first regulatory pathway driven by bile salts, the HNF4α-binding site at -826 to -814 is involved. Activation of FXR by bile salts or synthetic ligands leads to

a translocation to the nucleus and a competitive displacement of HNF4a from this binding site. The second pathway is mediated by FXR stimulation of fibroblast growth factor 19 (FGF-19) expression in the intestine. Fibroblast growth factor 19 migrates to the liver, binds to the FGF-receptor 4, and down-regulates apo(a) expression by mitogen-activated protein kinase (RAS-ERK1/2) signaling and binding of phosphorylated ELK-1 to the ETS promoter segment at 21603 to 21615 (Chennamsetty et al., 2011, Chennamsetty et al., 2012a).

Hepatocytes from primates synthesize a pre-form of apo(a) with a lower degree of glycosylation. Upon maturation, intracellular apo(a) reaches the Golgi and is released in a mature form as a glycoprotein, most probably without being linked to LDL. The genetically determined size of apo(a) correlates with the intracellular persistence time and thus, small isoforms are secreted much faster than large isoforms. This appears to be the reason for the inverse correlation between apo(a) size and plasma Lp(a) (Kostner et al., 2013, Frank et al., 1994) .

The assembly of Lp(a) from apo(a) and LDL is a two-step process and isn't dependent on any enzymatic activity. In the first step, specific K-IVs of apo(a), particularly K-IV types 3–6 non-covalently bind to lysine groups of apolipoprotein B in LDL, and This binding is reversible (Frank et al., 1995). In the second step, a covalent disulfide bond between Apo(a) cys4057 and apoB Cys4326 is formed (McCormick, 2004). Although the evidence for an extracellular assembly is preferred by many researchers, there are data that support an intracellular assembly (Dieplinger and Utermann, 1999). Alternative mechanisms are favored by Jenner et al. who supposed several dissociation and re-association steps between apo(a) and apoB containing lipoproteins (Jenner et al., 2005).

Lp(a) concentrations strongly and significantly correlated with the production rate, while there was no correlation with Lp(a) catabolism (Kronenberg et al., 2006). The liver is the major organ of Lp(a) degradation (Lawn et al., 1995). In vivo approximately 50% of Lp(a) is taken up by the liver, followed by the kidney, the spleen, and muscles (Kronenberg et al., 1997). In the urine of healthy humans apo(a) is

found as free apo(a) fragments which are 50–160 kD in size. It is not fully clear where and how these fragments are formed, but it appears that a large portion is formed extra-renally, and is selectively excreted by the kidney (Kostner et al., 1996).

1.5.3. Genetics of lipoprotein (a):

The apo(a) gene location is on chromosome 6q26-q27and is one of the most polymorphic genes in humans (Kostner et al., 2013). Different types of isoforms are independently associated with Lp(a) concentrations. Apo(a) occurs in isoforms differing in their sizes. The size heterogeneity of apo(a) is attributable to different numbers of exon sequences encoding kringle IV type 2 repeats and correlates with plasma Lp(a) levels, whereby small isoforms give rise to high plasma Lp(a) and vice versa (Utermann et al., 1987). Mechanistically, larger apo(a) isoforms are most likely trapped and degraded immediately following biosynthesis in the rough endoplasmic reticulum or in the Golgi at a higher rate than smaller ones (White et al., 1993a). There is a variable number of a pentanucleotide repeats (TTTTA) in the promoter region of the apo(a) gene (Kamstrup et al., 2008), and the untranslated region of the gene contains +93 C/T polymorphism (Valenti et al., 1999). Further mutations and polymorphisms are abundant in the apo(a) gene and explain in part the variable concentrations of plasma Lp(a) (Clarke et al., 2009, Li et al., 2011).

There are two functional SNPs in the distal enhancer region 20 kB upstream of the apo(a) gene, and numerous polymorphisms in the K-IV domains of the apo(a) gene impacting significantly on apo (a) concentrations (Ichinose, 2008).

In human plasma, Lp(a) concentrations range from < 1 to 250 mg/dL and more (Kostner et al., 2013). Approximately one-third of the variation in Lp(a) concentrations is due to genetic heterogeneity of the Lp(a) locus(Lanktree et al., 2010, Li et al., 2011). The upstream proximal apo (a) promoter region contains numerous regulatory elements including response elements for interleukin 6 (IL-6), HNF-1 and -4, cAMP response element-binding protein, and sterol regulatory element binding proteins (Ramharack et al., 1998, Schultz et al., 2010, Kostner et al., 2013). Lp(a)

concentrations vary significantly among different ethnic groups for example, Caucasians have much lower plasma concentrations than African Americans independent of isoform variations. In Chinese and some Asian populations, the opposite is true (Ogorelkova et al., 2001, Geethanjali et al., 2003).

1.5.4. Non-genetic factors affecting lipoprotein (a):

Lp(a) levels can be increased in certain disease conditions such as renal disease. Both nephrotic syndrome and renal failure increase Lp(a) levels two- to three-fold, probably by different mechanisms. While in the nephrotic syndrome the rate of Lp(a) biosynthesis appears increased, reduced Lp(a) catabolism may occur in renal failure. Urinary apo(a) significantly falls once the glomerular filtration rate becomes < 70 ml/min and this contributes to the increased plasma Lp(a) in chronic kidney disease (Kostner et al., 1998a, Kostner et al., 1998b).

Since Lp(a) synthesis occurs in the liver, it is not surprising that Lp(a) is decreased in liver diseases such as cholestasis. Other substances that are toxic to the liver including alcohol and several drugs have also been shown to significantly decrease Lp(a) (Kostner et al., 2013). It has been found that hypothyroid patients had significantly higher and hyperthyroid patients had lower Lp(a) concentrations than normal subjects (Erem et al., 1999).

Results of studies investigating Lp(a) levels in patients with type 1 diabetes mellitus (DM) are inconsistent. No correlation has been found in young children with type 1 DM and it is clear that the positive correlation with type 1 diabetes in adults is mostly indirect, because many patients at the same time may have renal failure (Kollerits et al., 2006). The pathogenesis of type 2 diabetes is complex and it isn’t expectable that Lp(a) levels are affected in all patients to the same extent. This may explain the highly conflicting results in which unaltered, higher, or lower Lp(a) plasma concentrations have been reported (Mora et al., 2010). The cross-sectional analysis of plasma Lp(a) concentrations from 36 studies reported that Lp(a) levels were 11% lower in diabetic patients than normal subjects (Collaboration, 2009). Although the type of diabetes was

undetermined, it is likely that 90% of these diabetic patients were affected by type 2 diabetes (Qi and Qi, 2012).

Lp(a) levels have an inverse relationship with fasting insulin, and insulin and glucose levels measured by the 2-h glucose challenge test in both diabetic and nondiabetic subjects (Rainwater and Haffner, 1998). A report based on an in vitro study showed that insulin suppresses apoA production in primary cynomolgus monkey hepatocytes (Neele et al., 1999). These data may partly explain the reduced Lp(a) levels in patients with type 2 diabetes (characterized by hyperinsulinemia and insulin resistance), and elevated Lp(a) levels in patients with type 1 diabetes (characterized by insulin deficiency (Qi and Qi, 2012).

Several pharmacologic agents have been shown to influence plasma Lp(a) levels. For example, lipid-lowering drugs, including niacin, statins, fibrates, neomycin, and ezetimibe have all been reported to decrease plasma Lp(a). Unfortunately, their effects are inconsistent. The only drug that consistently reduces Lp(a) by up to 35% by interfering with apo(a) gene transcription is niacin or its derivatives (Chennamsetty et al., 2012b). Regular aspirin intake has also been reported to lower apoA mRNA expression by up to 85% (Kagawa et al., 1999).

Other agents have been demontrated to lower Lp(a) levels, including ascorbic acid combined with l-lysine, l-carnitine, calcium antagonists, angiotensin converting enzyme inhibitors, androgens, estrogen and its replacements (e.g., tibolone), antiestrogens (e.g., tamoxifen) and thyroxine replacement (Tziomalos et al., 2009, Nordestgaard et al., 2010, Dubé et al., 2012).

The administration of insulin in patients with type 2 diabetes led to higher plasma Lp(a) levels than the treatment with diet or oral hypoglycemic agents (Heller et al., 1993). Also in type 1 diabetes insulin administration results in a significant increase in Lp(a) levels (Matsumoto et al., 1997). Metformin, an antihyperglycemic drug, has been reported to decrease Lp(a) levels in nondiabetic individuals, while a study in patients with type 2 diabetes showed that Lp(a) levels were not influenced by

metformin administration (Ovalle and Bell, 1999, Tack et al., 1999, Lanktree et al., 2010).

1.5.5. Mechanisms of Lp(a) atherogenicity:

Many in vitro studies have shown that Lp(a) is involved in atherogenesis by a number of different mechanisms (Deb and Caplice, 2004). Lipoprotein(a) increases the expression of adhesion molecules (e.g. VCAM-1) and stimulates human vascular endothelial cells to produce monocyte chemotactic protein (MCP), resulting in monocyte adhesion and migration (Allen et al., 1998). After transfer into the arterial intima, Lp(a) may be more avidly trapped than LDL as it binds to the extracellular matrix not only through apolipoprotein B, but also via its apolipoprotein (a) component, thereby contributing its cholesterol content to the expanding atherosclerotic plaque (Nielsen, 1999).

An in vitro studies showed that, Lp(a) binds to several extracellular matrix proteins including fibrin (Lundstam et al., 1999) and defensins, which are released by neutrophils during inflammation and severe infection, and provide a link between Lp(a) and the extracellular matrix (Bdeir et al., 1999).

Lp(a) reduces the activation of latent transforming growth factor- (TGF-) by displacing plasminogen from the surfaces of macrophages in atherosclerotic plaques. In the absence of activated TGF- , cytokines might stimulate smooth muscle cell proliferation and the differentiation into more atherogenic cellular phenotype (Grainger et al., 1994). In addition, lipoprotein(a) has been shown to carry modified phospholipids implicated in vascular inflammation and atherosclerosis (Edelstein et al., 2003, Navab et al., 2002).

The protease domain of apolipoprotein (a) contains 88% amino acid identity to the protease domain of plasminogen, but is not an active protease able to degrade fibrin (McLean et al., 1987). On the contrary, lipoprotein (a) prevents the activation of plasminogen by t-PA (the enzyme which cleaves the plasminogen to form plasmin) (Marcovina and Koschinsky, 2003) partly by competing with plasminogen for binding

to the fibrin or endothelial cell surface (Hajjar et al., 1989, Loscalzo et al., 1990), and also stimulates endothelial cell synthesis of plasminogen activator inhibitor (PAI-1) possibly hindering fibrinolysis (Etingin et al., 1991). Recently, lipoprotein(a) has been demonstrated to bind and inhibit tissue factor pathway inhibitor (TFPI), a potent inhibitor of the tissue factor mediated coagulation cascade, thus perhaps directly promoting thrombosis (Caplice et al., 2001).

Some studies showed that Lp(a) particles are sensitive to oxidation and that the increased risk of cardiovascular diseases associated with elevated plasma Lp(a) levels may be due in part to their oxidative modification and pick up by macrophages, resulting in the formation of foam cells (Naruszewicz et al., 1992). The oxidative form of Lp(a) (ox-Lp(a)) might reduce the fibrinolytic activity through the reduction of plasminogen activation by t-PA, and the enhancement of vascular endothelial cells to produce PAI-1, and might impair endothelium-dependent vasodilation (Malaguarnera et al., 2012). Nonenzymatically glycated Lp(a) is higher in diabetic patients than normal subjects. Glycation of Lp(a) further enhances the production of PAI-1 and attenuates the synthesis of t-PA in arterial and venous endothelial cells (EC). The formation of advanced glycation end-products (AGEs) and EC-mediated oxidative modification may contribute significantly to the alterations of the production of PAI-1 and t-PA induced by Lp(a). The combination of hyperglycemia and hyperlipoprotein (a) may attenuate EC-derived fibrinolytic activity, which may promote the development of thrombosis and atherosclerosis in subjects with diabetes (Doucet et al., 1995, Lawn et al., 1992).

1.6. Aim of study:

Diabetes mellitus causes a two-fold higher risk for a wide range of vascular diseases, independent of other known risk factors. Any additional risk factor along with diabetes would increase the vascular disease risk. High Lp(a) levels had been proven to be a risk factor for atherosclerosis and related morbidity and mortality in many studies. It would be logical to consider higher vascular disease risk among diabetic patients with elevated Lp(a) levels although such an association is yet to be proven in controlled trials.

Type 2 diabetics are usually hyperinsulinemic and insulin tends to lower the Lp(a) levels. Large population-based studies have shown inverse associations between Lp(a) levels and the incidence of diabetes. However, some Arabic studies clearly showed higher Lp(a) levels among type 2 diabetics. These conflicting reports on the association between Lp(a) levels and type 2 diabetes prompted us to estimate the Lp(a) levels in Libyan type 2 diabetic patients, to correlate the values with other lipid parameters, and to examine the relationship to insulin, glycemic control, body mass index, and blood pressure.

Chapter 2

Subjects and Methods

2. Subjects and methods

2.1. Subjects:

A total of 100 Libyan patients with type 2 DM (55 women and 45 men) were recruited from the Benghazi Center for Diagnosis and Treatment of Diabetes, and Alhaia Clinic in Benghazi, and 30 apparently healthy age and sex- matched individuals (16 women and 14 men) were selected from the High Institute of Medical Professions-Benghazi, to serve as controls.

Informed consent was obtained from all subjects before the study. The diagnosis of diabetes was based on previous history of diabetes based on the American Diabetes Association criteria 2006 (i.e., A1c $\geq$ 6.5%, or fasting plasma glucose level $\geq$ 126 mg/dL, or 2-h plasma glucose $\geq$ 200 mg/dl during an oral glucose tolerance test). Clinical information and medical history were obtained through the review of patient medical files and patients' interviews. Face-to- face interview were based on a questionnaire (Appendix I), that included variables such as age, sex, date of the diagnosis, physical activity, history of smoking, following a diet, and any health problems, or prescriptions.

The height and weight were measured and obesity was defined as body mass index (BMI) of $\geq$ 30 kg/ m^2, where BMI was calculated by dividing the weight in kilograms on height in meters squared. Blood pressure was measured using mercury sphygmomanometer with subjects in a seated position. Systolic and diastolic pressures were determined at the time of the appearance and disappearance of Korotkoff sounds, respectively.

All patients presented stable metabolic conditions. Patients presenting any disease that could affect their metabolic status and the parameters studied such as nephrotic syndrome, acute or chronic renal failure, liver disease, thyroid disorders, acute infections, stroke, gout, diabetic ketoacidosis and non-ketotic hyperosmolar syndrome were excluded. The patients with history of familial hypercholesterolemia or acute myocardial infarction were also excluded from the study. The history of medication

was recorded and the patients taking insulin, lipid lowering agents, oral contraceptives, calcium antagonist, beta blockers, and steroids were also excluded.

The control group consisted of healthy subjects who were not suffering from any acute infection, or metabolic or psychological disorder. They were non-smoker, and non-overweight. They had no history of familial hypercholesterolemia or DM.

The diabetic subjects were divided into good and poor glycemic control groups based on a cutoff HbA_{1c} value of 7.5%.

2.2. Methods:

2.2.1. Blood collection:

Venous blood samples were drawn from all the participants after at least 10 hours fasting. Blood was collected in EDTA and plain tubes, and sera were separated from plain tubes and stored at – 70^{o}C until the assays were performed. The whole blood was stored at 4-8^{o}C, and analyzed for HbA1c within a week. Sera were analyzed for Lp(a), insulin, blood glucose, total cholesterol, triacylglycerol, and HDL.

2.2.2. Instruments:

The measurements of glucose, HbA1c, total cholesterol (TC), triacylglycerol(TAG), high density lipoprotein (HDL), and LDL were done using the standard procedures and available commercial kits in a fully automated system COBAS INTEGRA 400 plus (ROCH, Germany).

Serum insulin was measured using COBAS e 411 (ROCH, Germany).

Serum Lp(a) was estimated using Microplate Washer and Reader (LINEAR, Spain).

2.2.3. Estimation of blood glucose (FBG):

Test principle:

Enzymatic reference method with hexokinase.

Hexokinase (HK) catalyzes the phosphorylation of glucose by ATP to form glucose-6-phosphate and ADP. A second enzyme, glucose-6-phosphate dehydrogenase (G6PDH) is used to catalyze the oxidation of glucose-6-phosphate by $NADP^+$ to form NADPH.

$$\text{D-glucose} + \text{ATP} \xrightarrow{hexokinase} \text{D-glucose-6-phosphate} + \text{ADP}$$

$$\text{D-glucose-6-phosphate} + NADP^+ \xrightarrow{GDPDH} \text{D-6-phosphogluconate} + \text{NADPH} + H^+$$

The concentration of the NADPH formed is directly proportional to the glucose concentration. It is determined by measuring the increase in absorbance at 340 nm.

Test definition:

Measuring mode: Absorbance

Abs. calculation mode: Endpoint

Reaction mode: R-S

Reaction direction: Increase

Test range: 0-730 mg/dl

Wavelength A/B: 340/409 nm

Test temperature: 25 ^{o}C or 37 ^{o}C

Calculation:

COBAS INTEGRA analyzer automatically calculate the analyte concentration of each sample using the calculation formula:

Sample. Conc = Stand. Abs/ Sample. Abs × Stand. Conc.

(Roche Diagnostics, 2007).

2.2.4. Estimation of serum total cholesterol (TC):

Test principle:

Enzymatic colorimetric method:

Cholesterol esters are cleaved by the action of cholesterol esterase to yield free cholesterol and fatty acid. Cholesterol oxidase then catalyzes the oxidation of cholesterol to cholest-4-en-3-one and hydrogen peroxide. In the presence of hydrogen peroxide, peroxidase enzyme catalyzes the oxidative coupling of phenol and 4-aminoantipyrine to form a red quinine-imine dye.

$$\text{Cholesterol ester} + H_2O \xrightarrow{\textit{cholesterol esterase}} \text{cholesterol} + \text{fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\textit{cholesterol oxidase}} \text{cholest-4-en-3-one} + H_2O_2$$

$$2\ H_2O_2 + \text{4-aminoantipyrine} + \text{phenol} \xrightarrow{\textit{peroxidase}} \text{quinine-imine dye} + 4\ H_2O$$

The color intensity of the dye formed is directly proportional to the cholesterol concentration. It is determined by measuring the increase in absorbance at 512 nm.

Test definition:

Measuring mode: Absorbance

Abs. calculation mode: Endpoint

Reaction mode: R-S

Reaction direction: Increase

Test range: 0-800 mg/dl

Wavelength A/B: 512/659 nm

Test temperature: 25 ^{o}C or 37 ^{o}C

Calculation:

COBAS INTEGRA analyzer automatically calculate the analyte concentration of each sample using the calculation formula:

Sample. Conc = Stand. Abs/ Sample. Abs × Stand. Conc.

(Roche Diagnostics, 2007).

2.2.5. Estimation of serum triacylglycerols (TAG):

Test principle:

The lipase enzyme hydrolyses triacylglycerol sequentially to di and monoacylglycerol and finally to glycerol. Glycerol Kinase (GK) using ATP as PO_4 source converts glycerol liberated to glycerol-3-phosphate (G-3-phosphate). G-3-phosphate oxidase (GPO) oxidizes G-3-phosphate and forms dihydroxy acetone phosphate and hydrogen peroxide. Peroxidase (POD) uses the hydrogen peroxide formed to oxidize 4-aminophenazone and 4-chlorophenol to a colored complex.

$$\text{Triacylglycerol} + H_2O \xrightarrow{lipoprotein\ lipase} \text{glycerol} + \text{fatty acids}$$

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

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{glycerophosphate\ oxidase} \text{dihydroxyacetone phosphate} + H_2O_2$$

$$H_2O_2 + \text{4-aminophenazone} + \text{4-chlorophenol} \xrightarrow{peroxidase} \text{4-(p-benzoquinone-monoimino)-phenazone} + 2H_2O + HCL$$

The absorbance of the colored complex is measured at 512 nm which is proportional to triacylglycerol concentration.

Test definition:

Measuring mode: Absorbance

Abs. calculation mode: Endpoint

Reaction mode: R-S

Reaction direction: Increase

Wavelength A/B: 512/659 nm

Test temperature: 25 ^{o}C or 37 ^{o}C

Calculation:

COBAS INTEGRA analyzer automatically calculate the analyte concentration of each sample using the calculation formula:

Sample. Conc = Stand. Abs/ Sample. Abs × Stand. Conc.

(Roche Diagnostics, 2007).

2.2.6. Estimation of serum high density lipoprotein-cholesterol (HDL-c):

Test principle:

Homogenous enzymatic colorimetric assay.

In the presence of magnesium sulfate and dextran sulfate, water-soluble complexes with LDL, VLDL, and chylomicrons are formed which are resistant to PEG-modified enzymes. The cholesterol concentration of HDL-cholesterol is determined enzymatically by cholesterol esterase and cholesterol oxidase coupled with PEG to the amino groups (approximately 40%). Cholesterol esters are broken down into free cholesterol and fatty acid by cholesterol esterase. In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to Δ4-cholestenone and hydrogen peroxide. In the presence of hydrogen peroxide, peroxidase enzyme catalyzes the oxidative coupling of HSDA and 4-aminoantipyrine to form a purple blue dye.

$$\text{HDL-cholesterol ester} + H_2O \xrightarrow{PEG-cholesterol\ esterase} \text{HDL-cholesterol} + \text{fatty acid}$$

$$\text{HDL-cholesterol} + O_2 \xrightarrow{PEG-cholesterol\ esterase} \Delta 4\text{-cholestenone} + H_2O_2$$

$$2H_2O_2 + \text{4-aminoantipyrine} + \text{HSDA} + H^+ \xrightarrow{peroxidase} \text{Purple blue pigment} + 4H_2O$$

The color intensity of the coloured dye formed is directly proportional to the HDL-cholesterol concentration. It is determined by measuring the increase in absorbance at 583 nm.

Test definition:

Measuring mode: Absorbance

Abs. calculation mode: Endpoint

Reaction mode: R1-S-SR

Reaction direction: Increase

Wavelength A/B: 583/659 nm

Test temperature: 25 ^{o}C or 37 ^{o}C

Calculation:

COBAS INTEGRA analyzer automatically calculate the analyte concentration of each sample using the calculation formula:

Sample. Conc = Stand. Abs/ Sample. Abs × Stand. Conc.

(Roche Diagnostics, 2007).

2.2.7. Calculation of low density lipoprotein-cholesterol (LDL-c):

COBAS INTEGRA analyzer calculate LDL-cholesterol concentration of each sample using the Friedwalds' formula.

LDL-c = (TC – HDL-c) – TAG/5.

2.2.8. Estimation of glycosylated hemoglobin (HbA1c):

Test principle:

Turbidimetric inhibition immunoassay.

The whole blood sample is hemolyzed using a detergent-containing reagent. The hemolyzing step can either be performed automatically on the instrument or manually using hemolyzing reagent. The liberated hemoglobin in the hemolyzed sample is

converted to a stable derivative which is measured photometrically during the preincubation phase of the immunological reaction. Glycohemoglobin (HbA1c) in the sample reacts with the anti-HbA1c antibody to form soluble antigen-antibody complexes. The polyhaptens in the reagent react with excess anti-HbA1c antibodies and form an insoluble antibody-polyhapten complex. This complex can be measured turbidimetrically, the higher the HbA1c concentration, the lower the turbidity.

Hb Test definition:

Measuring mode: Absorbance

Abs. calculation mode: Endpoint

Reaction mode: R1-S

Reaction direction: Increase

Wavelength A/B: 378/659 nm

Test temperature: 25 ^{o}C or 37 ^{o}C

HbA1c Test definition:

Measuring mode: Absorbance

Abs. calculation mode: Endpoint

Reaction mode: R1-S-SR

Reaction direction: Increase

Wavelength A/B: 340/659 nm

Test temperature: 25 ^{o}C or 37 ^{o}C

Calculation:

COBAS INTEGRA analyzer automatically calculate the analyte concentration of each sample using the calculation formula:

$$\text{HbA1c. Conc} = (\text{HbA1c/HB}) \times 100.$$

(Roche Diagnostics, 2007).

2.2.9. Estimation of serum insulin:

Test principle:

Electrochemiluminescence.

Electrochemiluminescence (ECL) processes are known to occur with numerous molecules including compounds of ruthenium, osmium, rhenium or other elements. ECL is a process in which highly reactive species are generated from stable precursors at the surface of an electrode. These highly reactive species react with one another producing light. The development of ECL immunoassays is based on the use of a ruthenium chelate as the complex for the development of light. The chemiluminescent reactions that lead to the emission of light from the ruthenium complex are initiated electrically rather than chemically. This is achieved by applying a voltage to the immunological complexes (including the ruthenium complex) that are attached to Streptavidin – coated micro particles.

The chemiluminescent insulin assay employs two monoclonal antibodies which together are specific for human insulin (**sandwich principle**).

- In the first step, patient sample is combined with a reagent containing biotinylated insulin antibody and a ruthenium-labelled insulin specific antibody in an assay cup. During a nine minute incubation step, antibodies capture the insulin present in the sample and form sandwich complexes.
- In the second step, streptavidin – coated paramagnetic particles are added. During a second nine minute incubation, the biotinylated antibody attaches to the streptavidin – coated surface of micro particles.
- After the second incubation, the reaction mixture containing the immune complexes is transported into the measuring cell; the immune complexes are magnetically entrapped on the working electrode, but unbound reagent and sample are washed away by the system buffer.
- In the ECL reaction, the conjugate is a ruthenium based derivative and the chemiluminescent reaction is electrically stimulated to produce light. The

amount of light produced is directly proportional to the amount of insulin present in the sample.

Test definition:

Sample volume: 20 µl

Detection limit: 0.20 µU/ml

Measuring range: 0.20 - 1000 µU/ml

Test temperature: 37 ^{o}C

Calculation:

COBAS INTEGRA analyzer automatically calculate the analyte concentration of each sample.

(Roche Diagnostics, 2009).

2.2.10. Assay of Lipoprotein (a):

2.2.10.1. Principle:

Abcam's Lipoprotein A (APOA) human in vitro ELISA employs a quantitative **sandwich** enzyme immunoassay technique.

A polyclonal antibody specific for human lipoprotein A has been precoated onto 96-well plates and blocked. Lipoprotein A in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for lipoprotein A, which is recognized by streptavidin-peroxidase conjugate. All unbound material is then washed away, and TMB is added to visualize streptavidin-peroxidase enzymatic reaction. TMB is catalyzed by streptavidin-peroxidase to produce a blue color product that changes into yellow after adding acidic stop solution. The density of yellow coloration is directly proportional to the amount of lipoprotein A captured in plate.

2.2.10.2. Reagent preparation:

All reagents were diluted, brought to room temperature, and freshly used. Reagent bottles were mixed gently to remove any crystals formed.

Diluent (10X): The diluent was prepared via dilution 1:10 with distilled water.

Sample: The serum samples were diluted 1:8000 with diluent as follows: 5µl of sample were added to 395µl of diluent (1:80) to make solution A; then 10µl of solution A were added to 990µl of diluent (1:100) to make a final working solution (1:8000).

Standard curve: The lipoprotein A standard was reconstituted with the appropriate amounts of diluent to generate a standard solution of 50 ng/ml. The standard was allowed to sit for 10 minutes with gentle agitation prior to making dilutions. Duplicate standard points were prepared by serially diluting the standard solution (50 ng/ml) 1:2 with equal volume of diluent to produce 25, 12.5, 6.25, 3.123, 1.56, and 0.78 ng/ml. diluent served as the zero standard (0 ng/ml).

Biotinylated Human Lipoprotein A Antibody: The biotinylated antibody was agitated gently to be diluted 1:50 with the desired amount of diluent.

Wash Buffer Concentrate (20x): The wash buffer was diluted 1:20 with the appropriate volume of distilled water.

SP Conjugate (100x): The conjugate was agitated gently, and diluted 1:100 with the appropriate amount of diluent.

2.2.10.3. Procedure:

1- 50 µl sample or standard were added to each well in the plate. The plate was covered with a sealing tape and incubated for two hours in room temperature.
2- Each well in the plate was washed six times with 300 µl wash buffer using microplate washer, and then the plate was inverted to decant the content of the wells and tapped 4-5 times on absorbent paper towel to completely remove the liquid.
3- 50 µl of biotinylated human lipoprotein A antibody were added to each well, and the plate was incubated for one hour.
4- The microplate was washed again as describe above.
5- 50 µl of streptavidin-peroxidase conjugate were added to each well, and the plate was incubated for 30 minutes.
6- The plate was washed for the last time as described above.

7- During washing, the microplate reader was turned on, and the program for lipoprotein (a) assay was selected.

8- 50 µl of chromogen were added to each well, and the microplate was incubated for about 10 minutes.

9- The plate was agitated gently to ensure thorough mixing, and the bubbles in the wells were broken with pipette tip.

10- 50 µl of stop solution were added to each well. The color changed from blue to yellow.

11- The absorbance of each well in the plate was immediately measured on microplate reader at 450 nm.

2.2.10.4. Calculation:

Calculations of Lp(a) concentrations were performed automatically by the microplate reader.

The standard curve is constructed by the instrument via plotting the absorbance of standards (Y) at 450 nm against log of the known concentrations of standards (X), and the results are reported as concentration of Lp (a) in samples (Figure 2.1).

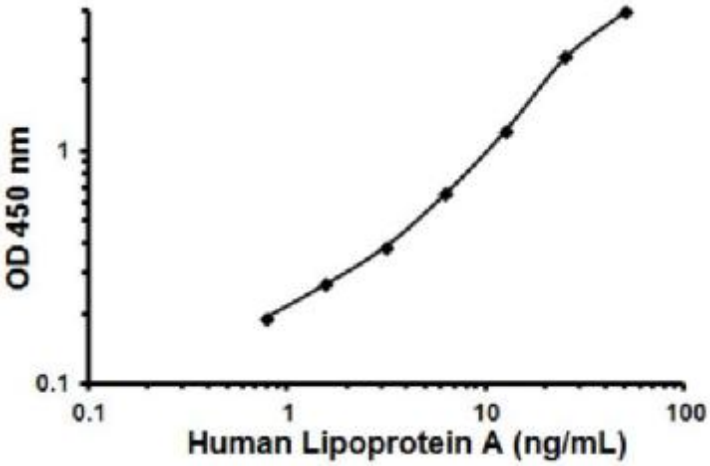
The concentrations of Lp (a) calculated from the standard curve were multiplied in their respective dilution factor, because samples have been diluted prior to the assay.

Unit conversion factor: ng/ml * *0.0001* = mg/dl.

2.2.10.5. Sensitivity:

The minimum detectable dose of lipoprotein A is typically 0.8 ng/mL.

(Abcam, 2013)



http://www.abcam.cn/lipoprotein-a-apoa-human-elisa-kit-ab108878-protocols.html.

Figure 2.1: Standard curve.

2.3. Statistical analysis:

The data were analyzed using the statistical package for the social sciences (SPSS version 17). Descriptive characteristics of the study participants were calculated as mean ± standard deviation (SD). Analysis of variance (ANOVA) was used to determine differences in subject characteristics. Pearson's correlation coefficient determination was done to evaluate the degree of association between Lp (a) and clinical and biochemical parameters. *P* value (two- tailed) < 0.05 was considered as statistically significant.

Chapter 3

Results

3. Results

The mean age and standard deviation (SD) of the patients with type 2 DM selected for this study was 50.17 ± 5.16, and the female: male ratio was 11: 9. The age range was 37- 63 years. The mean age and SD of the healthy control subjects was 47.33 ± 6.18, and the female: male ratio was 8: 7. The age range was 37-60 years.

3.1. Body mass index (BMI):

BMI was significantly higher in both groups of diabetics when compared to the normal control group ($p < 0.05$). There was a significant difference in BMI between controlled and uncontrolled diabetic groups ($p = 0.009$) (Table 3.1, Figure 3.1).

Table 3.1: Mean ± SD of body mass index (BMI) in diabetic and healthy control subjects.

groups	Uncontrolled patients N= 54	Controlled patients N= 46	Controls N= 30	*P* value
BMI (Kg/m^2)	**26.8 ± 0.88**	**26.34 ± 1.03**	**24.48 ± 0.32**	**0.00**

N: number of patients.

Uncontrolled patients: Diabetic patients with poor glycemic control (HbA1c > 7.5%).

Controlled patients: Diabetic patients with good glycemic control (HbA1c ≤ 7.5%).

Controls: Normal healthy subjects.

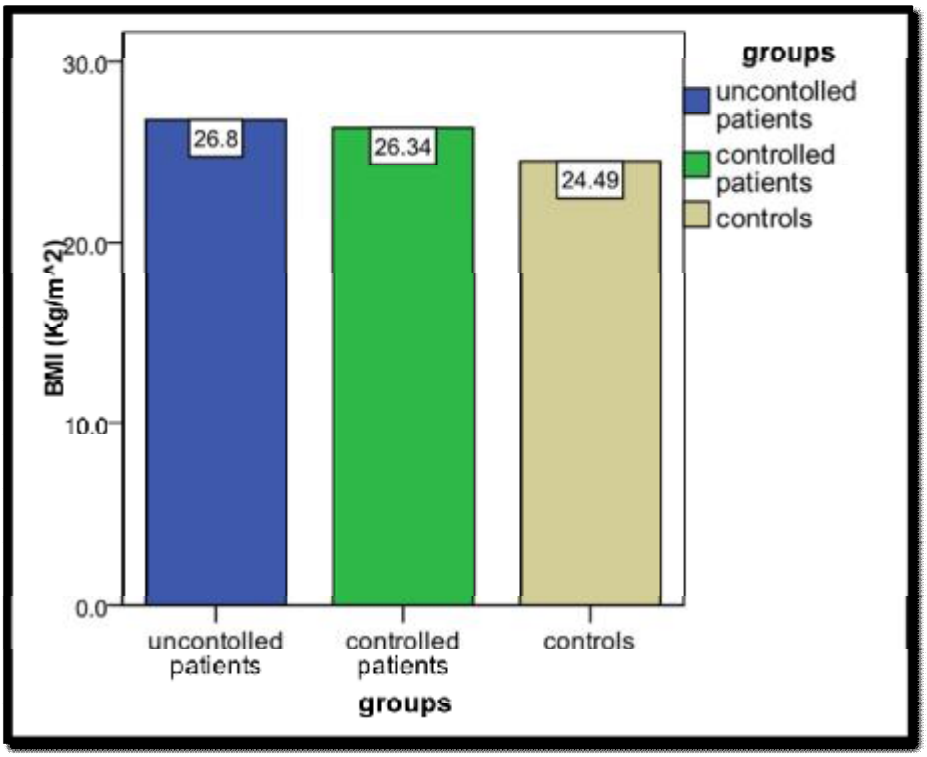


Figure 3.1: Mean of body mass index (BMI) in diabetic and healthy control subjects.

3.2. Blood Pressure (BP):

Systolic and diastolic blood pressure measurements showed significant differences comparing diabetic patients to the control group ($p < 0.05$). Non-significant difference in systolic and diastolic blood pressure measurements were found between controlled and uncontrolled diabetic groups (p= 0.87 and p= 0.88, respectively) (Table 3.2, Figure 3.2).

Table 3.2: Mean ± SD of blood pressure (BP) in diabetic and healthy control subjects.

Groups	Uncontrolled patients N= 54	Controlled patients N= 46	Controls N= 30	*P* value
Diastolic BP (mm Hg)	84.65 ± 4.75	84.78 ± 4.48	76.09 ± 3.7	0.00
Systolic BP (mm Hg)	132.72 ± 8.36	132.98 ± 8.1	114.1 ± 6.83	0.00

N: number of patients.

Uncontrolled patients: Diabetic patients with poor glycemic control (HbA1c > 7.5%).

Controlled patients: Diabetic patients with good glycemic control (HbA1c ≤ 7.5%).

Controls: Normal healthy subjects.

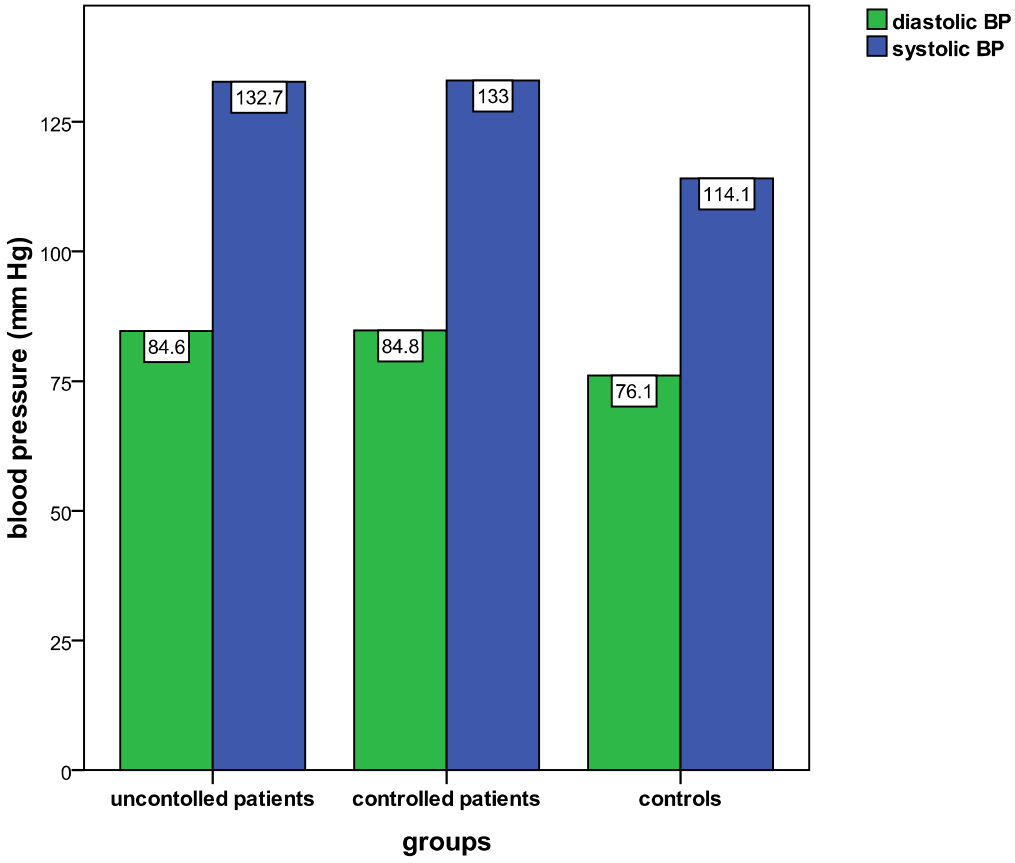


Figure 3.2: Mean of blood pressure (BP) in diabetic and healthy control subjects.

3.3. Fasting blood Glucose (FBG):

Diabetic patients had significantly higher blood glucose than non-diabetic controls ($p > 0.05$). In addition, diabetics with poor glycemic control had significantly higher levels of blood glucose when compared to patients with good glycemic control ($p= 0.00$) (Table 3.3, Figure 3.3).

Table 3.3: Mean ± SD of fasting blood glucos (FBG) in diabetic and healthy control subjects.

groups	Uncontrolled patients N= 54	Controlled patients N= 46	Controls N= 30	*P* value
FBS (mg/dl)	185.1 ± 62.7	129.93 ± 38	89.4 ± 5.56	0.00

N: number of subjects.

Uncontrolled patients: Diabetic patients with poor glycemic control (HbA1c > 7.5%).

Controlled patients: Diabetic patients with good glycemic control (HbA1c ≤ 7.5%).

Controls: Normal healthy subjects.

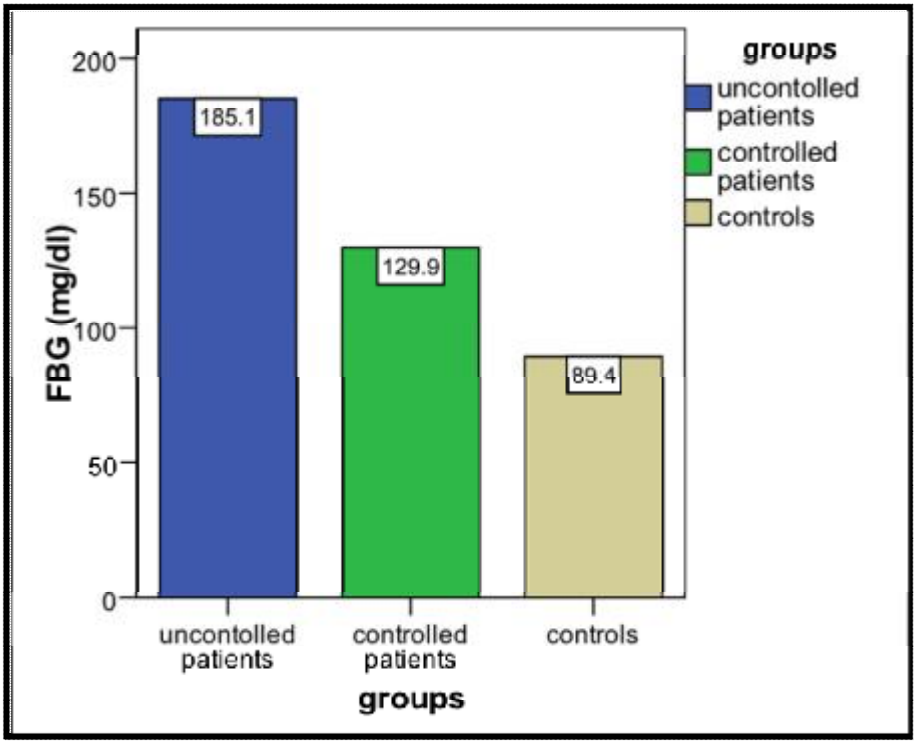


Figure 3.3: Mean of fasting blood Glucose (FBG) in diabetic and healthy control subjects.

3.4. Glycosylated hemoglobin (HbA_{1C}):

Diabetic patients had a significant increase in HbA_{1C} when compared to normal control subjects ($p < 0.05$) (Table 3.4, Figure 3.4).

Table 3.4: Mean ± SD of HbA_{1C} in diabetic and healthy control subjects.

groups	Uncontrolled patients N= 54	Controlled patients N= 46	Controls N= 30	*P* value
HbA1c (%)	9.48 ± 1.3	6.41 ± 0.73	4.88 ± 0.27	0.00

N: number of subjects.

Uncontrolled patients: Diabetic patients with poor glycemic control (HbA1c > 7.5%).

Controlled patients: Diabetic patients with good glycemic control (HbA1c ≤ 7.5%).

Controls: Normal healthy subjects.

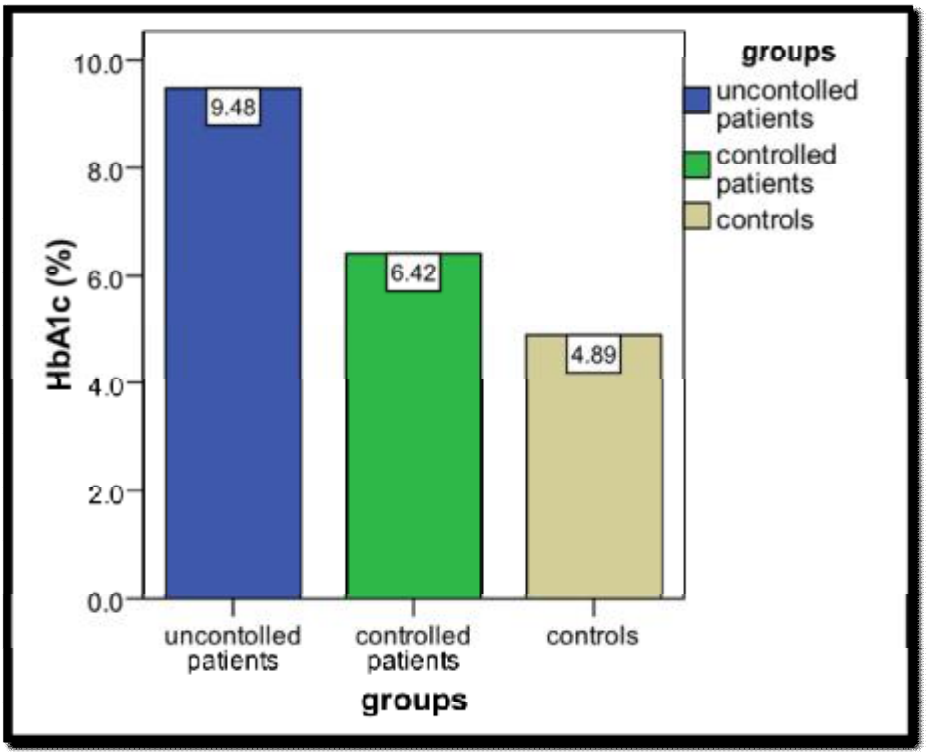


Figure 3.4: Mean of glycosylated hemoglobin (HbA_{1C}) in diabetic and healthy control subjects.

3.5. Serum total cholesterol (TC):

The mean level of the serum total cholesterol in patients with type 2 DM was higher than that of the control subjects and the difference was statistically significant ($p < 0.05$). Comparing total serum cholesterol in controlled and uncontrolled diabetic patient groups showed no significant differences (p= 0.64) (Table 3.5, Figure 3.5).

Table 3.5: Mean ± SD of total cholesterol (TC) in diabetic and healthy control subjects.

groups	Uncontrolled patients N= 54	Controlled patients N= 46	Controls N= 30	*P* value
TC (mg/dl)	225.61 ± 44	222.39 ± 31.34	163 ± 17.56	0.00

N: number of subjects.

Uncontrolled patients: Diabetic patients with poor glycemic control (HbA1c > 7.5%).

Controlled patients: Diabetic patients with good glycemic control (HbA1c ≤ 7.5%).

Controls: Normal healthy subjects.

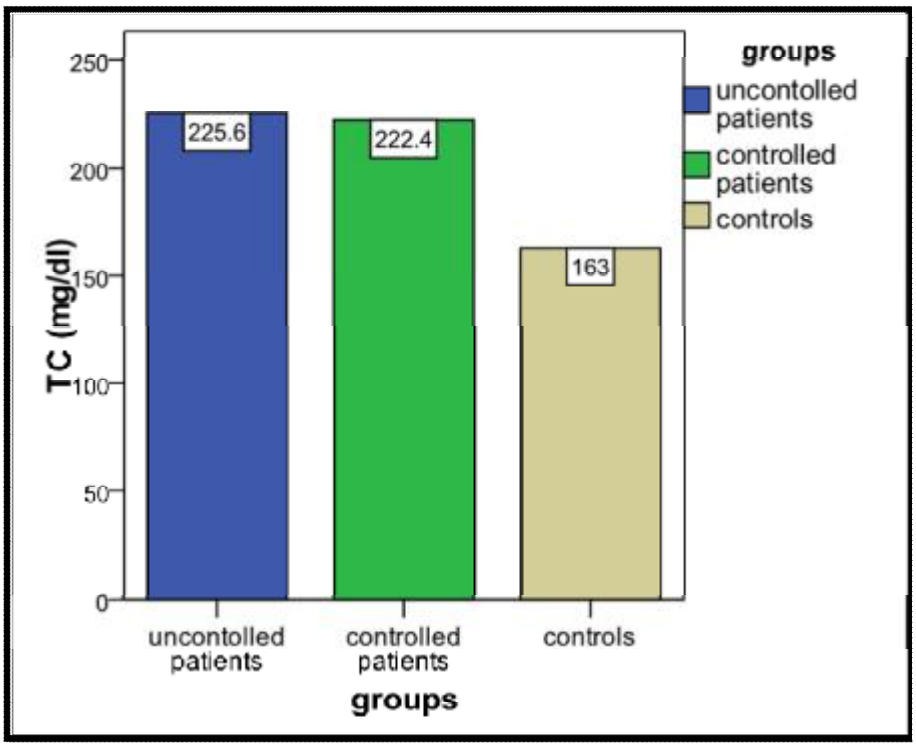


Figure 3.5: Mean of Total cholesterol (TC) in diabetic and healthy control subjects.

3.6. High density lipoprotein-cholesterol (HDL-c):

Patients with type 2 DM had significantly lower HDL-c than normal control subjects ($p < 0.05$). There was a non-significant difference in HDL-c between controlled and uncontrolled diabetic groups (p= 0.28) (Table 3.6, Figure 3.6).

Table 3.6: Mean ± SD of high density lipoprotein-cholesterol (HDL-c) in diabetic and healthy control subjects.

groups	Uncontrolled patients N= 54	Controlled patients N= 46	Controls N= 30	*P* value
HDL-c (mg/dl)	44.69 ± 11.37	46.83 ± 9.86	70.18 ± 6.77	0.00

N: number of subjects.

Uncontrolled patients: Diabetic patients with poor glycemic control (HbA1c > 7.5%).

Controlled patients: Diabetic patients with good glycemic control (HbA1c ≤ 7.5%).

Controls: Normal healthy subjects.

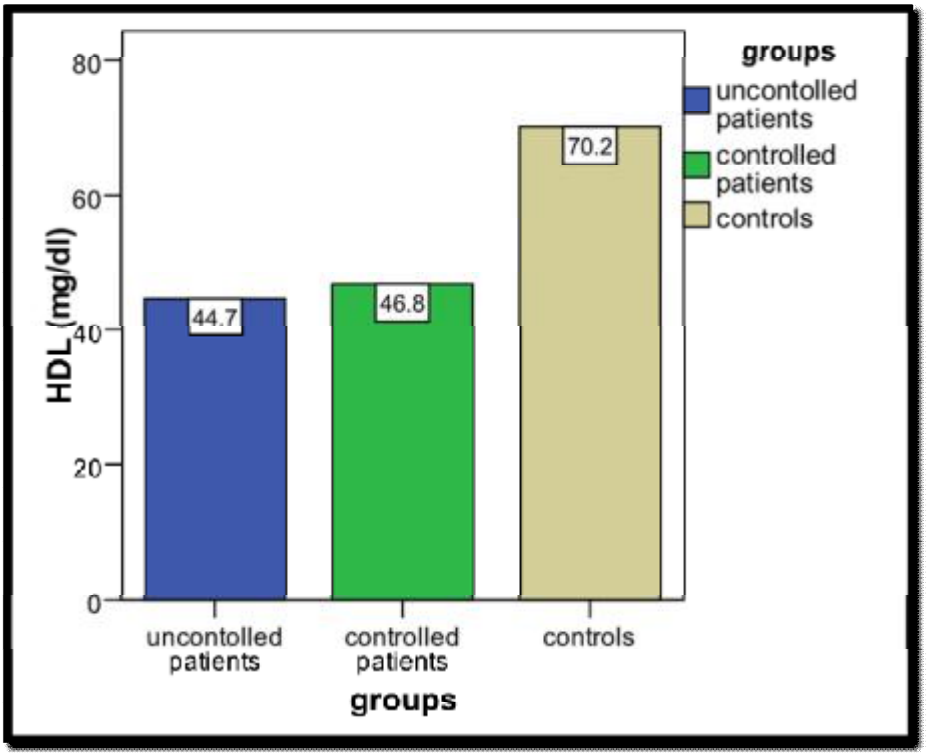


Figure 3.6: Mean of high density lipoprotein-cholesterol (HDL-c) in diabetic and healthy control subjects.

3.7. Low density lipoprotein-cholesterol (LDL-c):

LDL-c was significantly higher in both groups of diabetic patients when compared to the normal control group ($p < 0.05$). The difference between LDL-c in controlled and uncontrolled diabetic patient groups was statistically insignificant ($p= 0.62$) (Table 3.7, Figure 3.7).

Table 3.7: Mean ± SD of low density lipoprotein-cholesterol (LDL-c) in diabetic and healthy control subjects.

groups	Uncontrolled patients N= 54	Controlled patients N= 46	Controls N= 30	*P* value
LDL-c (mg/dl)	139.53 ± 43.67	142.97 ± 33.88	91.51 ± 14.25	0.00

N: number of subjects.

Uncontrolled patients: Diabetic patients with poor glycemic control (HbA1c > 7.5%).

Controlled patients: Diabetic patients with good glycemic control (HbA1c ≤ 7.5%).

Controls: Normal healthy subjects.

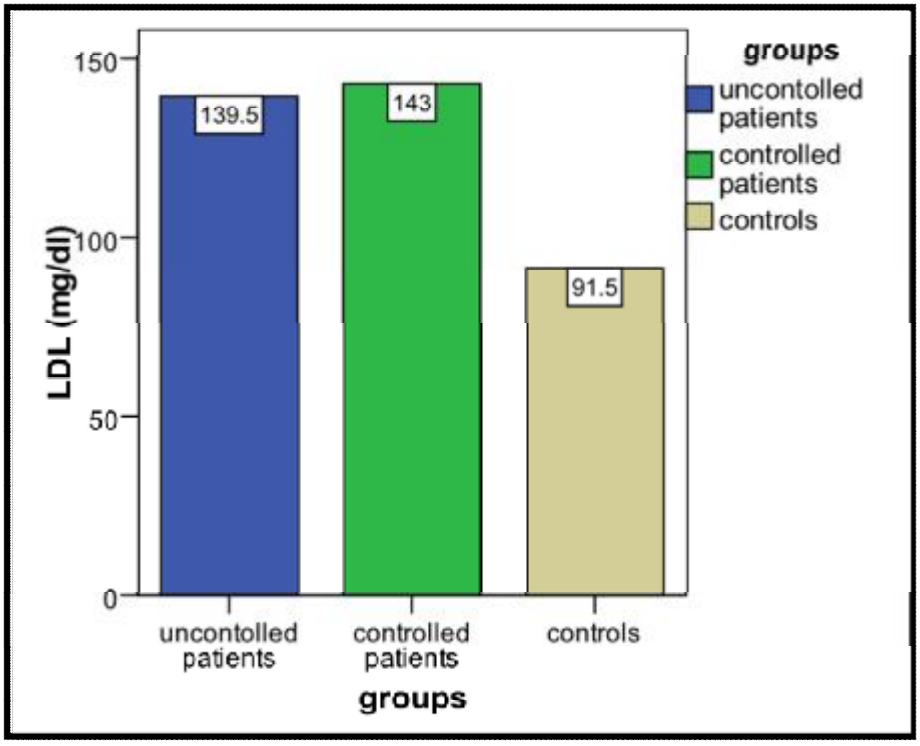


Figure 3.7: Mean of low density lipoprotein-cholesterol (LDL-c) in diabetic and healthy control subjects.

3.8. LDL-c/HDL-c Ratio:

Diabetic patient groups had a significantly higher LDL/HDL ration when compared to the normal control group ($p < 0.05$). There was a non-significant difference in LDL/HDL ratio between controlled and uncontrolled diabetic groups ($p = 0.56$) (Table 3.8, Figure 3.8).

Table 3.8: Mean ± SD of LDL-c/HDL-c ratio in diabetic and healthy control subjects.

groups	Uncontrolled patients N= 54	Controlled patients N= 46	Controls N= 30	*P* value
LDL/HDL Ratio	3.29 ± 1.27	3.17 ± 0.98	1.31 ± 0.26	0.00

N: number of subjects.

Uncontrolled patients: Diabetic patients with poor glycemic control (HbA1c > 7.5%).

Controlled patients: Diabetic patients with good glycemic control (HbA1c ≤ 7.5%).

Controls: Normal healthy subjects.

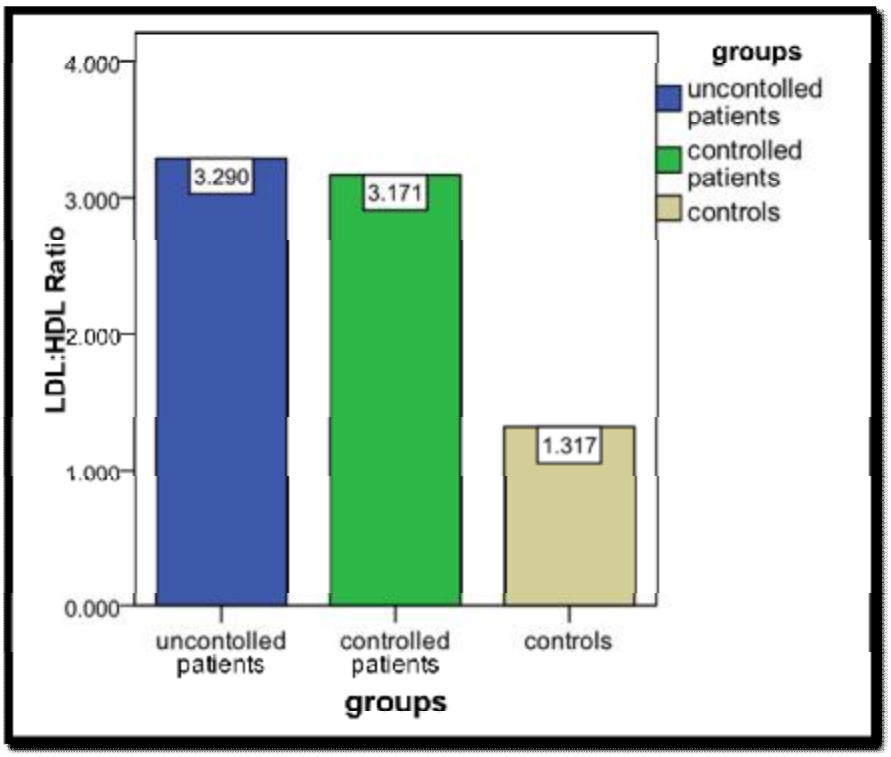


Figure 8: Mean of LDL-c/HDL-c ratio in diabetic and healthy control subjects.

3.9. Serum triacylglycerol (TAG):

Mean TAG levels were higher in the diabetic groups as compared to the control group and the difference was statistically significant ($p < 0.05$). Uncontrolled diabetic patient group had significantly higher levels of TAG than controlled diabetic patient group ($p = 0.003$) (Table 3.9, Figure 3.9).

Table 3.9: Mean ± SD of Triacylglycerol (TAG) in diabetics and healthy control subjects.

Groups	Uncontrolled patients N= 54	Controlled patients N= 46	Controls N= 30	*P* value
TAG (mg/dl)	205.62 ± 83.53	159.21 ± 78.24	109.5 ± 31.4	0.00

N: number of subjects.

Uncontrolled patients: Diabetic patients with poor glycemic control ($HbA_{1C} > 7.5\%$).

Controlled patients: Diabetic patients with good glycemic control ($HbA_{1C} \leq 7.5\%$).

Controls: Normal healthy subjects.

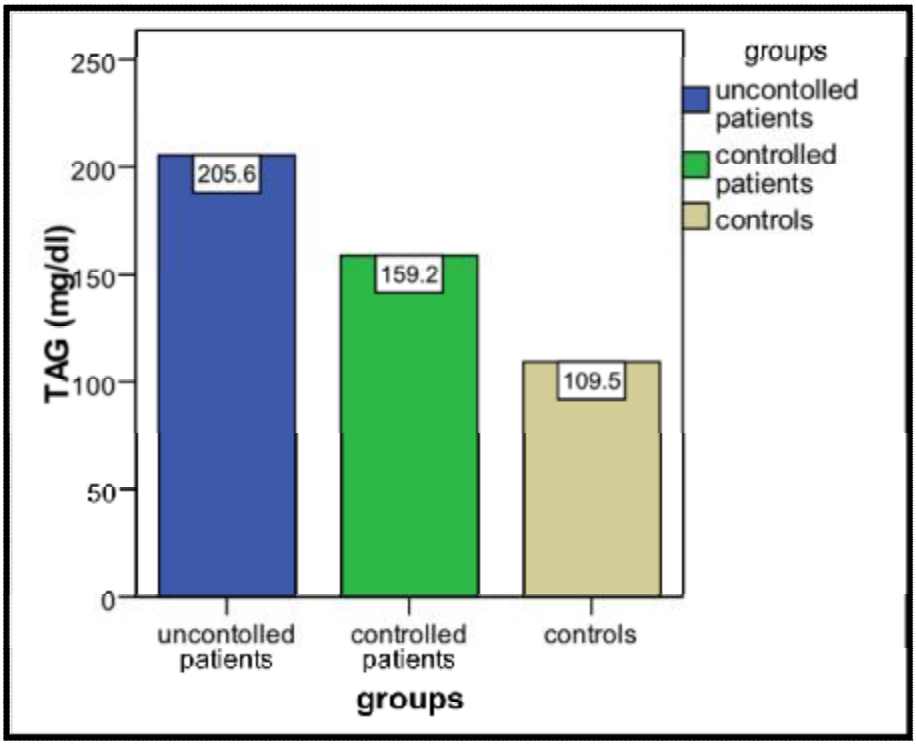


Figure 3.9: Mean of triacylglycerol (TAG) in diabetic and healthy control subjects.

3.10. Serum Insulin:

Serum insulin levels were significantly higher in both groups of diabetic patients when compared to the normal control group ($p < 0.05$). The controlled diabetic patient group showed a non-significant difference in serum insulin when compared to the uncontrolled diabetic patient group ($p = 0.19$) (Table 3.10, Figure 3.10).

Table 3.10: Mean ± SD 0f serum insulin in diabetic and healthy control subjects.

groups	Uncontrolled patients N= 54	Controlled patients N= 46	Controls N= 30	*P* value
Insulin (µIU/ml)	16.11 ± 11	13.54 ± 10.3	8.32 ± 4.2	0.003

N: number of subjects.

Uncontrolled patients: Diabetic patients with poor glycemic control (HbA1c > 7.5%).

Controlled patients: Diabetic patients with good glycemic control (HbA1c ≤ 7.5%).

Controls: Normal healthy subjects.

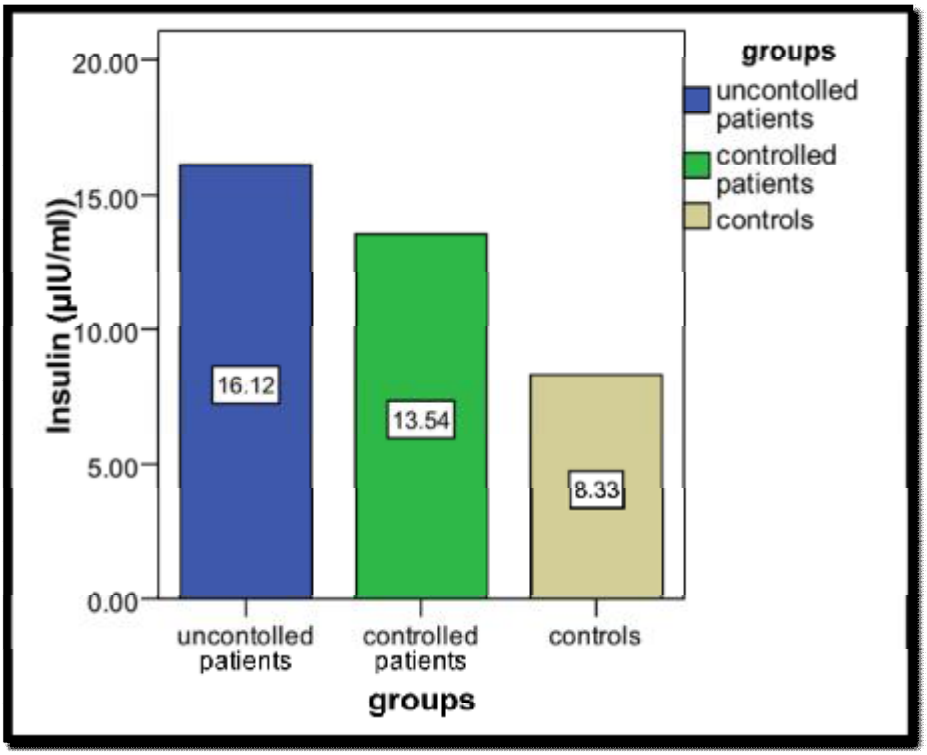


Figure 3.10: Mean of serum insulin in diabetic and healthy control subjects.

3.11. Lipoprotein (a):

The mean levels of Lp(a) in type 2 diabetic patient groups were significantly higher than that of the normal control group ($p < 0.05$). The difference in Lp(a) level between the controlled and uncontrolled diabetic patient groups was statistically insignificant ($p = 0.30$) (Table 3.11, Figure 3.11). The mean Lp(a) levels in both males and females were comparable (males vs females- 11.3 mg/dl vs 12.2 mg/dl, $p = 0.29$). In addition, no significant differences were observed in Lp(a) levels in relation to age.

Table 3.11: Mean ± SD of Lipoprotein (a) in diabetic patients and healthy control subjects.

groups	Uncontrolled patients N= 54	Controlled patients N= 46	Controls N= 30	*P* value
Lp(a) (mg/dl)	11.4 ± 4.2	12.25 ± 4.9	8.13 ± 2	0.00

N: number of subjects.

Uncontrolled patients: Diabetic patients with poor glycemic control (HbA1c > 7.5%).

Controlled patients: Diabetic patients with good glycemic control (HbA1c ≤ 7.5%).

Controls: Normal healthy subjects.

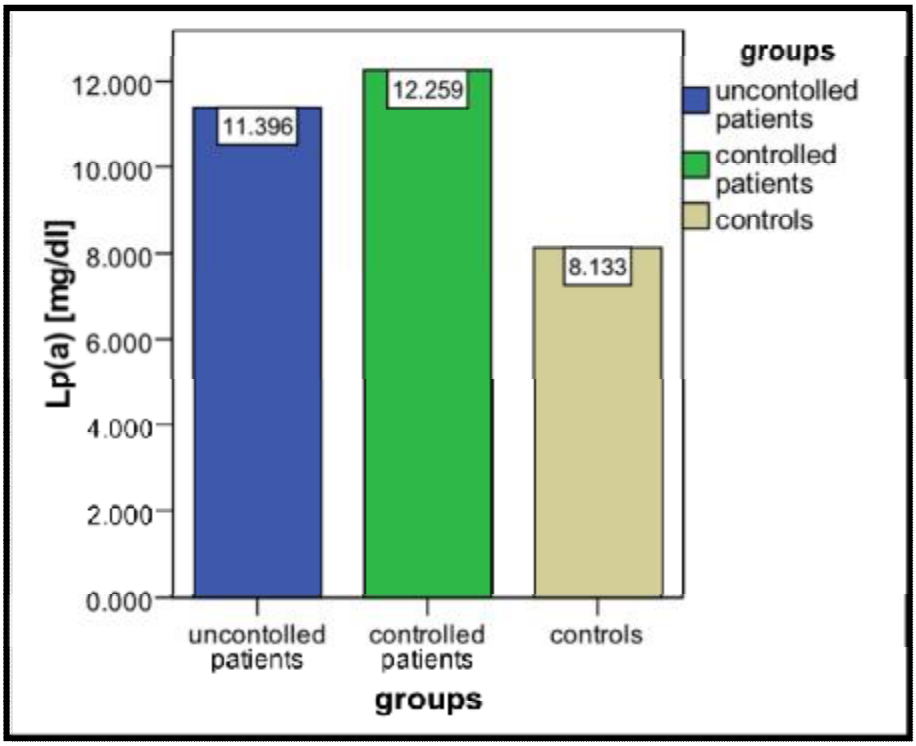


Figure 3.11: Mean of lipoprotein (a) in diabetic and healthy control subjects.

3.12. Relationship between Different Parameters

Pearson's correlation analysis of BMI, BP, lipids, Lp (a), and glycemic control parameters in diabetic patients showed that, BMI was positively correlated with systolic BP (r= 0.267, $p < 0.01$), diastolic BP (r= 0.303, $p < 0.01$), FBS (r= 0.338, $p < 0.01$), HbA1c (r= 0.278, $p < 0.01$), and TAG (r= 0.676, $p < 0.01$), and negatively correlated with LDL-c (r= - 0.327, $p < 0.01$). There was a linear and positive correlation between systolic BP and TC (r= 0.430, $p < 0.01$), and between systolic BP and LDL-c (r= 0.311, $p < 0.01$). There was a positive correlation between diastolic BP and TC (r= 0.434, $p < 0.01$), and between diastolic BP and LDL-c (r= 0.236, $p < 0.05$). FBS concentration was positively correlated with HbA_{1C} (r= 0.745, $p < 0.01$), and TAG (r= 0.230, $p < 0.05$). There was a strong positive relationship between TC and LDL-c (r= 0.740, $p < 0.01$). Furthermore, TC was positively correlated with LDL-c/HDL-c ratio (r= 0.729, $p < 0.01$), and LDL-c positively correlated with TC/HDL-c ratio (r= - 0.419 , $p< 0.01$). TAG was negatively correlated with HDL-c (r= - 0.471, $p < 0.01$), and LDL-c (r= - 0.456 , $p < 0.01$). On the other hand it had a positive correlation with TC/HDL-c ratio (r= 0.437, $p < 0.01$). Insulin levels had a strong positive correlation with insulin/glucose ratio (r= 0.973, $p < 0.01$).

Lp(a) was positively correlated with TC (Figure 3.12), and LDL-c (Figure 3.13). No significant correlation was seen between Lp(a) and, age, BMI, BP, TAG, HDL-c, LDL-c/HDL-c ratio, TC/HDL-c ratio, insulin and the parameters of glycemic control (HbA1c and fasting glycemia).

Table 3.12 summarize correlation among LP(a) and some parameters in type 2 diabetic patients.

In non-diabetic control subjects, Lp(a) levels did not show any significant correlations with BMI, BP, lipid profile, insulin, or glycemic control parameters.

Table 3.13 shows the non-significant correlations between Lp(a) and some parameters in non-diabetic control subjects.

Table 3.12: Correlation (r) between Lp(a) and some clinical and biochemical parameters in type 2 diabetic patients.

Variable	Pearson correlation (r)	*P* value
Age	0.154	0.127
BMI	- 0.044	0.663
Systolic BP	0.051	0.613
Diastolic BP	0.103	0.306
FBS	0.025	0.802
HbA1c	- 0.001	0.990
TC	0.309**	0.002
HDL-c	0.042	0.680
LDL-c	0.236*	0.018
TAG	- 0.013	0.900
Insulin	0.040	0.694
LDL-c/HDL-c	0.163	0.105
TC /HDL-c	0.142	0.157

**. Correlation is significant at the 0.01 level.

*. Correlation is significant at the 0.05 level.

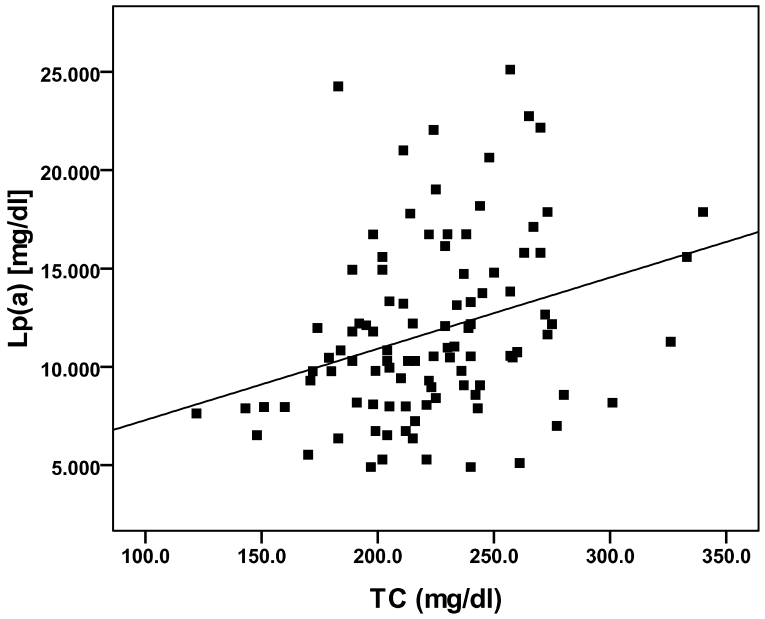


Figure 3.12: correlation between Lp(a) and TC in diabetic group.

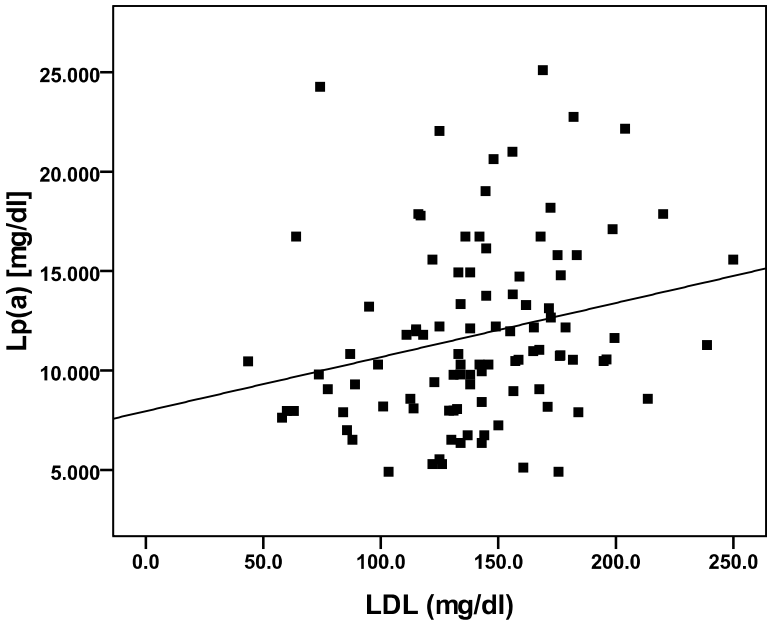


Figure 3.13: correlation between Lp(a) and LDL-c in diabetic group.

Table 3.13: Correlation (r) between Lp(a) and some clinical and biochemical parameters in non-diabetic healthy control subjects.

Variable	Pearson correlation (r)	*P* value
Age	- 0.101	0.595
BMI	0.006	0.976
Systolic BP	- 0.476	0.022
Diastolic BP	- 0.345	0.107
FBS	- 0.430	0.018
HbA1c	- 0.045	0.815
TC	- 0.094	0.622
HDL-c	0.220	0.243
LDL-c	- 0.247	0.188
TAG	0.140	0459
Insulin	0.256	0.173

Chapter 4

Discussion

4. Discussion

In the present case-control study of 100 type 2 diabetic patients and 30 normal control subjects, Lp(a) was significantly higher in diabetic patients when compared to control subjects. Lp(a) was positively correlated with TC, and LDL-c. No significant correlations were found between Lp(a) and glycemic control parameters, insulin, TAG, HDL-c, BMI, and BP.

BMI was significantly higher in diabetic group than control group, and this finding is consistent with several similar studies (Turner et al., 1998a, Daousi et al., 2006, El-Hazmi and Warsy, 1999). It is well documented that obesity increases morbidity and mortality in type 2 diabetic patients and short-term studies have demonstrated that even moderate weight reduction through diet and/or exercise can improve patient hyperglycemia (Maggio and Pi-Sunyer, 2003, Scheen, 2003). However, the few long-term cohort studies regarding the effects of weight loss on glycemic control in diabetic patients produced inconsistent results (Aucott et al., 2004, Shoff et al., 1998). It has been speculated that the inconsistencies may have arisen from the confounding influences of other factors on body weight, such as the disease process itself or the medications used (Heller, 2004, Turner et al., 1998a).

The present study showed that, BMI is positively correlated with SBP, and DBP in type 2 diabetic group. This result is consistent with other studies of type 2 diabetic patients conducted in India, and Taiwan (Tseng, 2007, Dudekhula et al., 2012).

The association between BMI and BP has been widely reported across general populations in Africa, Asia, Latin America, United States, and Canada. In a study that included five Latin American populations (urban) and seven Asian populations (four urban, three rural), direct positive relationships of similar magnitude were observed between BMI and BP, despite differences in mean BMI levels between the study populations (Tesfaye et al., 2006, Collaboration, 2004, Nogueira et al., 1996).

A study by Tseng reported that, in type 2 diabetic patients without hypertension history, BMI increase of 1kg/m^2 corresponded to an increase in DBP of 0.45 and 0.46

mmHg in men and women, respectively; and a respective increase in SBP of 0.62 and 0.64 mmHg (Tseng, 2007). These were comparable to the findings of the study reported by Kissebah and Krakower, they documented a gain of 1 unit BMI corresponding to an increase in SBP of approximately 0.59 and 0.80mmHg in men and women, respectively (Kissebah and Krakower, 1994).

Based on our finding BMI showed a positive correlation with FBS. A positive correlation between BMI and fasting blood sugar in type 2 diabetics was previously reported by other studies (Al-mukhtar et al., 2006). These changes and correlations were explained by the statement put forward by DeFronozo and Ferrannini who reported that obesity causes peripheral resistance to insulin mediated glucose uptake and may also decrease the sensitivity of the beta-cells to glucose, so that obesity is associated with hyperglycemia (DeFronzo and Ferrannini, 1991).

The present study revealed a positive association between BMI and HbA1c in the diabetic group, and a significantly higher BMI mean value in diabetics with poor glycemic control (HbA1c > 7.5%) when compared with those with good glycemic control (HbA1c $\leq$ 7.5%). These findings are consistent with other studies (Habib, 2013, Daousi, 2006) that reported that the proportion of poorly controlled diabetics was increased with increasing BMI which means that poorly controlled diabetic patients were more in obese patients than non-obese patients.

A variety of intervention studies demonstrated that patients with type 2 diabetes who succeeded in losing weight often had modest improvements in glycemic control and cardiovascular risk profiles, as long as the weight loss is maintained (Gill, 2004).

BMI in the present study had a positive relationship with triacylglycerols in diabetic group. This positive correlation was found by many studies (Sone et al., 2007, Daousi et al., 2006). The most common lipid abnormality in diabetes is hypertriacylglycerolemia with or without increase in plasma cholesterol (Al-Nuaim et al., 1995), and BMI increase due to adiposity characterized by increased triacylglycerols and reduced HDL-c (Maki et al., 1997). A study carried out by Al-mukhtar et al. showed that diabetic patients with obesity have a significantly higher

levels of serum triacylglycerols when compared to non- obese diabetics (Al-mukhtar et al., 2006).

In the present study, BMI was negatively correlated with LDL-c in diabetic group.

Many studies showed a non-significant correlation between BMI and LDL-c (Himabindu et al., 2013, Sandhu et al., 2008), while other studies revealed a positive correlation between BMI and LDL-c (Al-mukhtar et al., 2006). LDL-c was determined by calculation using the Friedewald formula (cLDL-c). With the Friedewald equation, cLDL-c (in mg/dL) = TC – (HDL-c) – (TAG/5); a value of TAG dividing by 5 represents the VLDL-c concentration. An overproduction of the TAG-enriched large VLDL, a characteristic of diabetic dyslipidemia, leads to overestimation of the VLDL-c and underestimation of the cLDL-c concentrations, so that an increase in TAG is associated with a decrease in LDL-c by using friedwald formula (Srisawasdi et al., 2011), and this may explain the negative relation between LDL-c and BMI, which is strongly positively associated with serum triacylglycerols. A recent study has revealed significant differences in anthropometric parameters and lipid profile patterns in type II diabetics in three different ethnic groups living in Malaysia (Blebil et al., 2011). These studies indicate that anthropometric parameters and lipid profile patterns may vary from one ethnic group to another, one geographical region to another and among different races. So the results of one study cannot be extrapolated to other studies and generalized conclusions cannot be drawn for all populations.

The present study showed that systolic and diastolic blood pressures were significantly higher in diabetic group when compared to control group. Diabetic hypertensive patients were excluded from the study, because hypertension and its treatments affect serum Lp (a) levels. Many studies demonstrated a high prevalence of hypertension among diabetic patients. A study by Berraho et al. reported a prevalence of about 70.4 % (Berraho et al., 2012), while a study by Mengesha reported a prevalence of about 61.2 % (Mengesha, 2008).

In comparison with the general population, individuals with type 2 diabetes mellitus (DM) have a 2- to 4-fold increased risk for cardiovascular disease. Hypertension is an

important and modifiable risk factor for cardiovascular disease associated with DM, and the results of many studies suggested that 35% to 75% of the cardiovascular risk in diabetic patients can be attributed to the presence of hypertension (Booth et al., 2006, Lloyd-Jones et al., 2006, Lee et al., 2000).

In the present study, systolic and diastolic blood pressure of diabetic patients had significant positive correlation with serum cholesterol and LDL-c. Many studies concerned with type 2 diabetic population showed consistently similar results (Nasri et al., 2014, Behradmanesh and Nasri, 2012, Nasri, 2006). Elevated low-density lipoprotein cholesterol (LDL-c) may increase the sensitivity of angiotensin II. A study by Van Der Linde et al., on healthy young subjects with hypercholesterolemia showed increased sensitivity to angiotensin II that can be restored partly by LDLc- lowering therapy (van der Linde et al., 2006). These findings indicate that LDL-c levels directly influence angiotensin II sensitivity. In addition, it is possible that impaired endothelial function or up regulation of the AT1 receptor by low density lipoprotein cholesterol leads to an increase in diastolic blood pressure (Nishimura, 2012).

The present study results showed that FBS was significantly higher in diabetic patients when compared to the controls, and this finding is quite logical, since hyperglycemia is the primary factor that distinguishes diabetic individuals in general population. Furthermore, patients with HbA1c higher than 7.5% had significantly higher levels of FBS than patients with HbA1c $\leq$ 7.5%, and FBS showed a direct association with HbA1c. These findings are in agreement with the findings reported by others (VinodMahato et al., 2011, Rosediani et al., 2006, Ramona et al., 2011).

FBS in this study had a positive relationship with TAG, and this correlation had been reported in previous studies (VinodMahato et al., 2011, Poorsoltan et al., 2013). It has been documented that, insulin resistance in diabetes results in impaired glucose uptake and utilization by peripheral tissues, and impaired ability of insulin to suppress the expression or activity of gluconeogenic enzymes leading to hyperglycemia. This abnormality coexists with hypertriacylglycerolemia (Matsumoto et al., 2006), caused by the lack of insulin action on hepatic lipid metabolism, which normally limits the

synthesis of VLDL1, and on the lipolytic activity of lipoprotein lipase. Impaired insulin action results in defective suppression of lipolysis in adipose tissue with the influx of the released free fatty acids to the liver. This scenario induces a dramatic increase of VLDL1 production, the largest lipoproteins rich in triacylglycerols (Adiels et al., 2008, Krentz, 2003).

In the present study, HbA1c had a non-significant correlations with total cholesterol, LDL-c, HDL-c, and triacylglycerols, and these results are consistent with many other studies (Sheikhpour et al., 2013). In this study, patients with poor glycemic control (HbA1c $\geq$ 7.5%) showed a significantly higher levels of triacylglycerols when compared to those with good glycemic control (HbA1c < 7.5%). HbA1c had a positive correlation with TAG, but this correlation was statistically non-significant (p= 0.053). This finding is similar to the findings reported in many studies (VinodMahato et al., 2011, Rohlfing et al., 2002). Other studies reported that, HbA1c showed direct and significant correlation with cholesterol, triacylglycerols and LDL cholesterol, and reverse correlation with HDL cholesterol, and suggested HbA1c can predict serum lipid levels in both male and female diabetic patients (Blebil et al., 2011, Ramona et al., 2011, Gaurav et al., 2012). The level of HbA1c value $\leq$ 7.0% was said to be appropriate for decreasing the risk of cardiovascular complications (Rohlfing et al., 2002). Khan et al. reported that the severity of dyslipidemia increases in patients with higher HbA1c values. Elevated HbA1c and dyslipidemia are independent risk factors for CVD, therefore diabetic patients with elevated HbA1c and dyslipidemia can be considered as a very high risk group for CVD (Khan et al., 2007). Improving glycemic control can profoundly reduce the risk of cardiovascular events in diabetics. It has been estimated that reducing the HbA1c value by 0.2% could lower the mortality by 10% (Smith Jr, 2007).

The result of the present study revealed that serum total cholesterol, LDL cholesterol and triacylglycerols were significantly raised, whereas the level of HDL cholesterol was significantly lower in diabetics as compared to control subjects. These observed increases and decreases in serum lipid profile associated with diabetes mellitus are in

agreement with the findings of Çalışması, Kandula et al. , and Albriki et al. (Çalışması, 2008, Kandula and shegokar, 2013, Albrki et al., 2007).

LDL-c/HDL-c ratio in the present study was significantly higher in diabetic subjects than in the controls. Many studies showed consistent results (Gordon et al., 2010, Indumati et al., 2011). The LDL/HDL ratio is actually a more pure ratio than total cholesterol/HDL ratio, because LDL is a measure of "bad' cholesterol and HDL is a measure of "good" cholesterol, whereas the total cholesterol is the sum of HDL-c, LDL-c, and the VLDL-c. The LDL-c/HDL-c ratio reflects the bidirectional traffic of cholesterol entering and leaving the arterial intimae in a way that the individual levels of LDL-c and HDL-c do not (Kannel, 2005). Several large epidemiological and clinical studies have found the LDL-c/HDL-c ratio to be an excellent predictor of CHD risk (Packard et al., 2005).

The findings of this study showed a significant inverse relationship between TAG and HDL-c, and this relation is consistent with the findings of many studies(Mohammed et al., 2012, Kolovou et al., 2006). Under hypertriacylglycerolemic conditions characteristic of diabetes, there is excessive exchange of cholesterol esters and triacylglycerols between HDL and VLDL, mediated by cholesterol ester transfer protein (CETP). Hepatic lipase hydrolyzes TAG-rich HDL particles and, as a result HDL particles are rapidly catabolized and cleared from plasma. Reduced activity of LPL may further decrease the levels of HDL cholesterol by decreasing the conversion of HDL3 to HDL2 particles (Mooradian et al., 2008).

The present study also showed a negative correlation between TAG and LDL-c. Increased concentrations of VLDL particles in the circulation of patients with T2DM also increase the CETP-mediated exchange of cholesterol ester and triacylglycerol between VLDL and low density lipoprotein (LDL) cholesterol particles. This increase in triacylglycerol content of LDL particles makes them a good substrate for hepatic lipase, which hydrolyzes triacylglycerols in the LDL particles and increases their density, resulting in increased concentrations of small dense LDL particles (Krauss and Ronald, 1998, Krauss, 1998). Small dense LDL particles are highly atherogenic

because of their enhanced susceptibility to oxidative modification and increased uptake by the arterial wall (Brautbar and Ballantyne, 2011). Minnich et al. reported that, the CETP-mediated exchange of cholesterol ester and triacylglycerol between VLDL and low density lipoprotein (LDL) cholesterol particles was responsible for low LDL-c concentrations found under hypertriglyceredemic conditions (Minnich et al., 1989).

LDL-c in the present study was determined by calculation using the Friedewald formula (cLDL-c). With the Friedewald equation, cLDL-c (in mg/dL) = TC – (HDL-c) – (TAG/5); a value of TAG dividing by 5 represents the VLDL-c concentration. An overproduction of the TAG-enriched large VLDL, a characteristic of diabetic dyslipidemia, might lead to overestimation of the VLDL-c and underestimation of the cLDL-c concentrations, so that an increase in TAG is associated with a decrease in LDL-c by using friedwald formula (Srisawasdi et al., 2011).

Insulin in the present study was significantly higher in diabetic patients as compared to control subjects, and had a significant direct association with insulin resistance. These findings are in agreement with a study by Habib et al. (2009). Insulin secretion from pancreatic β cells increases in response to insulin resistance to maintain normal glucose levels (Stumvoll et al., 2005).

It has been observed that patients with type 2 DM have increased morbidity and mortality due to coronary risk events. This increased risk has been shown to be independent from conventional risk factors (Stamler et al., 1993). Different factors have been found to be responsible for an increased prevalence of CAD in T2DM. One of these are the raised serum levels of Lp(a) (Kostner and Kostner, 2002).

The major finding of the present study was revealing a significantly higher level of Lp(a) in diabetic patients when compared with control subjects. This significant finding is consistent with several studies (El-gari, 2013, Habib, 2013, Smaoui et al., 2004, Singla et al., 2009, Habib et al., 2009, Habib and Aslam, 2004, Patil et al., 2014).

The effect of hyperglycemia on the rate of synthesis, transcription, and translation of apo(a) is still not exactly known. The concentration of glycosylated Lp(a) is increased in the blood stream of diabetic patients. It is evident from many studies that glycosylation prolongs the half-life of lipoproteins and this may be applicable for Lp(a), which may result in higher levels of Lp(a) in diabetic patients (Klaya et al., 1997, Maca et al., 2007). Plasma Lp(a) concentrations are primarily regulated at the level of the gene that encodes apo(a), and an inverse correlation has been found between plasma Lp(a) concentration and apo(a) size that may result, at least in part, from the relatively inefficient secretion of the larger apo(a) isoforms from the hepatocytes. Additionally, the level of Lp(a) in human plasma is largely uninfluenced by diet, physical activity, and conventional lipid therapy (Marcovina and Koschinsky, 1999). Further supporting evidence in favor of the present study is the finding of Ribault et al. , who found that type 2 diabetic patients had a higher prevalence of low molecular weight isoforms of apo(a) (Ribault et al., 2000), raising the possibility that diabetic patients of the present study may have small size isoforms of apo(a), which result in higher levels of Lp(a).

Some studies found no differences in serum Lp(a) levels between diabetic patients and controls (Chang et al., 1995, Chico et al., 1996).

Rainwater, Hernandez and Albaharani demonstrated lower serum Lp (a) levels in diabetic patients as compared to control subjects. Rainwater et al. attributed their findings to the presence of large size Apo(a) isoforms responsible for lower plasma Lp(a) concentrations in type 2 diabetic patients. These authors presumed that nonenzymatic glycosylation of Apo(a) is responsible for its large size and each allele in diabetic subjects averaged 4.1 kDa larger than the same allele in nondiabetic subjects, which may be responsible for lower Lp(a) levels in type 2 diabetics (Rainwater et al., 1994, Hernández et al., 2001, AlBahrani et al., 2007).

The present study showed a non-significant correlation between either fasting glucose or HbA1c and plasma Lp (a). Changes in Lp (a) concentrations relative to diabetic control remain controversial, but most studies have failed to show any association of

Lp(a) levels with glycemic control in patients with type 2 DM (Smaoui et al., 2004, Singla et al., 2009, Habib and Aslam, 2004, Ogbera and Azenabor, 2010).

In the present study, Lp(a) was positively correlated with LDL-c, and total cholesterol. No significant correlation has been found between Lp(a) and TAG, HDL-c, BMI, or BP. These findings are in agreement with the results of many studies (Habib and Aslam, 2004, Smaoui et al., 2004, Habib and Aslam, 2003).

The positive correlation between Lp(a) and LDL-c, and total cholesterol is not surprising because dyslipidaemia is very common in T2DM. One possibility for elevated concentration of Lp(a) in diabetics could be explained by understanding the assembly of apo(a) with apo-B 100 moiety of LDL. Apo(a) is primarily synthesized in the liver and is entered into the circulation, where the LDL moiety is attached to it (White et al., 1993b). Thus, if higher number of LDL particles are present in the circulation, there would be more union between LDL and apo(a) and thus higher concentrations of Lp(a). But this cannot be the sole explanation for this association because the metabolic pathways of LDL and Lp(a) are quite different.

Some studies observed a negative correlation between Lp(a) and triacylglycerols (AlBahrani et al., 2007, Ogbera and Azenabor, 2010), and explained this relation by the fact that, apo(a) is present in triacylglycerol-rich particles (TRPs). Apo(a)-containing TRPs, in parallel with chylomicron remnants, would be rapidly endocytosed by the liver through the remnant-receptor pathway. Thus, the lower levels of Lp(a) in patients with hypertriacylglycerolemia could be the result of the rapid catabolism of TRP apo(a) compared with the slower apo(a) catabolism in the LDL density range (Hernández et al., 2001). Supportive evidence to this finding was reported by Ko et al. study, in which improving insulin resistance in T2DM subjects by Rosiglitazone (insulin sensitizing agent) was associated with decreasing of triacylglycerols and increasing of Lp(a) concentration (Ko et al., 2003).

Other studies revealed a negative relationship between Lp(a) and serum insulin, and speculated that higher Lp(a) levels among patients with a longer duration of type 2 diabetes may be related to lower plasma insulin levels in such individuals, and because

the direct relationship of vascular risk to the duration of diabetes (Habib et al., 2009, Carantoni et al., 1999). A report based on an in vitro study showed that insulin suppresses apoA production in primary cynomolgus monkey hepatocytes (Neele et al., 1999).This finding is inconsistent with the present studies, which showed a non-significant association between Lp(a) and serum insulin.

These results are in line with other studies that reported that CVD risk may be dependent on additional lipid risk factors and indicate that LDL-c may not be an independent risk factor for the development and progression of atherogenesis in type 2 DM. Lp(a) may to be a new member of metabolic syndrome and it may be useful in routine clinical practice in future as a cardiovascular risk marker.

The association of Lp(a) levels in DM has been a matter of some controversies. The major reasons for the inconsistent results of the prospective studies have been attributed to the variation in study design, collection and storage of samples, analytical techniques used, methods used for statistical analysis and population differences that reflect the known ethnic variability in the Apo(a) size isoforms and distribution of plasma Lp(a) levels.

Chapter 5

References

5. References

ABDUELKAREM, A., EL-SHAREIF, H. & SHARIF, S. 2010. Prognostic indices for hospital mortality among Libyan diabetic patients. *Practical Diabetes International,* 27, 392-395a.

ABCAM. 2013. Instructions for use ab 108878-lipoprotein A (APOA) Human ELISA Kit.

ADIELS, M., OLOFSSON, S.-O., TASKINEN, M.-R. & BORÉN, J. 2008. Overproduction of very low–density lipoproteins is the hallmark of the dyslipidemia in the metabolic syndrome. *Arteriosclerosis, thrombosis, and vascular biology,* 28, 1225-1236.

AL-MUKHTAR, S. B., AL-HMADANI, R. Y. & AL-NAEMI, A. A. H. 2006. obesity and lipid profile in type 2 diabetics. *Tikrit medical journal,* 12, 15-21.

AL-NUAIM, A. R., FAMUYIWA, O. & GREER, W. 1995. Hyperlipidemia among Saudi diabetic patients-pattern and clinical characteristics. *Annals of Saudi medicine,* 15, 240-240.

ALBAHRANI, A., ALKINDI, M., MARKS, E., ALYAHYAEE, S. & SHENKIN, A. 2007. Lipoprotein (a): an independent risk factor for ischemic heart disease that is dependent on triglycerides in subjects with type 2 diabetes mellitus. *Lipids Health Dis,* 6, 26.

ALBERTI, K. G. M. M. & ZIMMET, P. F. 1998. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO consultation. *Diabetic medicine,* 15, 539-553.

ALBRKI, W., ELZOUKI, A., EL-MANSOURY, A. & TASHANI, O. 2007. Lipid profiles in Libyan type II diabetics. *J Sci Appls,* 1, 18-23.

ALLEN, S., KHAN, S., TAM, S.-P., KOSCHINSKY, M., TAYLOR, P. & YACOUB, M. 1998. Expression of adhesion molecules by Lp (a): a potential novel mechanism for its atherogenicity. *The FASEB journal,* 12, 1765-1776.

ALMDAL, T., SCHARLING, H., JENSEN, J. S. & VESTERGAARD, H. 2004. The independent effect of type 2 diabetes mellitus on ischemic heart disease, stroke, and death: a population-based study of 13 000 men and women with 20 years of follow-up. *Archives of internal medicine,* 164, 1422-1426.

ANBER, V., GRIFFIN, B., MCCONNELL, M., PACKARD, C. & SHEPHERD, J. 1996. Influence of plasma lipid and LDL-subfraction profile on the interaction between low density lipoprotein with human arterial wall proteoglycans. *Atherosclerosis,* 124, 261-271.

AMERICAN DIABETES ASSOCIATION. 2012. Diagnosis and classification of diabetes mellitus. *Diabetes care,* 35, S64-S71.

AMERICAN DIABETES ASSOCIATION. 2011. Standards of medical care in diabetes—2011. *Diabetes Care,* 34, S11.

AUCOTT, L., POOBALAN, A., SMITH, W., AVENELL, A., JUNG, R., BROOM, J. & GRANT, A. 2004. Weight loss in obese diabetic and non-diabetic individuals and long-term diabetes outcomes–a systematic review. *Diabetes, Obesity and Metabolism,* 6, 85-94.

BARTER, P. & JONES, M. 1979. Rate of exchange of esterified cholesterol between human plasma low and high density lipoproteins. *Atherosclerosis,* 34, 67-74.

BARTER, P. J., BREWER, H. B., CHAPMAN, M. J., HENNEKENS, C. H., RADER, D. J. & TALL, A. R. 2003. Cholesteryl ester transfer protein a novel target for raising HDL and inhibiting atherosclerosis. *Arteriosclerosis, thrombosis, and vascular biology,* 23, 160-167.

BDEIR, K., CANE, W., CANZIANI, G., CHAIKEN, I., WEISEL, J., KOSCHINSKY, M. L., LAWN, R. M., BANNERMAN, P. G., SACHAIS, B. S. & KUO, A. 1999. Defensin promotes the binding of lipoprotein (a) to vascular matrix. *Blood,* 94, 2007-2019.

BEHRADMANESH, S. & NASRI, P. 2012. Serum cholesterol and LDL-c in association with level of diastolic blood pressure in type 2 diabetic patients. *J Ren Inj Prev,* 1, 23-6.
BELL, G. I. & POLONSKY, K. S. 2001. Diabetes mellitus and genetically programmed defects in β-cell function. *Nature,* 414, 788-791.
BERG, K. 1963. A new serum type system in man—the Lp system. *Acta Pathologica Microbiologica Scandinavica,* 59, 369-382.
BERRAHO, M., EL ACHHAB, Y., BENSLIMANE, A., RHAZI, K. E., CHIKRI, M. & NEJJARI, C. 2012. Hypertension and type 2 diabetes: a cross-sectional study in Morocco (EPIDIAM Study). *Pan African Medical Journal,* 11.
BLEBIL, A. Q., HASSAN, Y. & DUJAILI, J. A. 2011. Differences in demographics, lipid profile and other clinical characteristics among type 2 diabetic patients in the state of penang, Malaysia according to gender and races. *Asian J Pharm Clin Res,* 4, 130-3.
BODEN, G. 1997. Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. *Diabetes,* 46, 3-10.
BOOTH, G. L., KAPRAL, M. K., FUNG, K. & TU, J. V. 2006. Relation between age and cardiovascular disease in men and women with diabetes compared with non-diabetic people: a population-based retrospective cohort study. *The Lancet,* 368, 29-36.
BORÉN, J., OLIN, K., LEE, I., CHAIT, A., WIGHT, T. N. & INNERARITY, T. L. 1998. Identification of the principal proteoglycan-binding site in LDL. A single-point mutation in apo-B100 severely affects proteoglycan interaction without affecting LDL receptor binding. *Journal of Clinical Investigation,* 101, 2658.
BRAUTBAR, A. & BALLANTYNE, C. M. 2011. Pharmacological strategies for lowering LDL cholesterol: statins and beyond. *Nature Reviews Cardiology,* 8, 253-265.
ÇALIŞMASI, D. 2008. A study on lipid profile levels of diabetics and non-diabetics among Naini region of Allahabad, India. *Türk Biyokimya Dergisi [Turkish Journal of Biochemistry–Turk J Biochem],* 33, 138-141.
CAMONT, L., CHAPMAN, M. J. & KONTUSH, A. 2011. Biological activities of HDL subpopulations and their relevance to cardiovascular disease. *Trends in molecular medicine,* 17, 594-603.
CAPLICE, N. M., PANETTA, C., PETERSON, T. E., KLEPPE, L. S., MUESKE, C. S., KOSTNER, G. M., BROZE, G. J. & SIMARI, R. D. 2001. Lipoprotein (a) binds and inactivates tissue factor pathway inhibitor: a novel link between lipoproteins and thrombosis. *Blood,* 98, 2980-2987.
CARANTONI, M., ZULIANI, G., BADER, G., PALMIERI, E., VOLPATO, S., PASSARO, A., IMBASTARO, T., MEZZETTI, A. & FELLIN, R. 1999. Low density lipoprotein cholesterol, lipoprotein (a), and apo (a) isoforms in the elderly: relationship to fasting insulin. Associazione Medica Sabin. *Nutrition, metabolism, and cardiovascular diseases: NMCD,* 9, 228-233.
CHAHIL, T. J. & GINSBERG, H. N. 2006. Diabetic dyslipidemia. *Endocrinology and metabolism clinics of North America,* 35, 491-510.
CHANG, C.-J., KAO, J.-T., WU, T.-J., LU, F.-H. & TAI, T.-Y. 1995. Serum lipids and lipoprotein (a) concentrations in Chinese NIDDM patients: Relation to metabolic control. *Diabetes care,* 18, 1191-1194.
CHAWLA, A., REPA, J. J., EVANS, R. M. & MANGELSDORF, D. J. 2001. Nuclear receptors and lipid physiology: opening the X-files. *Science,* 294, 1866-1870.
CHENNAMSETTY, I., CLAUDEL, T., KOSTNER, K. M., BAGHDASARYAN, A., KRATKY, D., LEVAK-FRANK, S., FRANK, S., GONZALEZ, F. J., TRAUNER, M. & KOSTNER, G. M. 2011. Farnesoid X receptor represses hepatic human APOA gene expression. *The Journal of clinical investigation,* 121, 3724-3734.

CHENNAMSETTY, I., CLAUDEL, T., KOSTNER, K. M., TRAUNER, M. & KOSTNER, G. M. 2012a. FGF19 signaling cascade suppresses APOA gene expression. *Arteriosclerosis, thrombosis, and vascular biology,* 32, 1220-1227.

CHENNAMSETTY, I., KOSTNER, K. M., CLAUDEL, T., VINOD, M., FRANK, S., WEISS, T. S., TRAUNER, M. & KOSTNER, G. M. 2012b. Nicotinic acid inhibits hepatic APOA gene expression: studies in humans and in transgenic mice. *Journal of lipid research,* 53, 2405-2412.

CHICO, A., PÉREZ, A., CAIXÀS, A., ORDOÑEZ, J., POU, J. M. & DE LEIVA, A. Lipoprotein(a) concentrations and non-insulin-dependent diabetes mellitus: relationship to glycaemic control and diabetic complications. 1996. *Diabetes Research and Clinical Practice,* 33, 105-110.

CLARKE, R., PEDEN, J. F., HOPEWELL, J. C., KYRIAKOU, T., GOEL, A., HEATH, S. C., PARISH, S., BARLERA, S., FRANZOSI, M. G. & RUST, S. 2009. Genetic variants associated with Lp (a) lipoprotein level and coronary disease. *New England Journal of Medicine,* 361, 2518-2528.

CLAY, M. A., NEWNHAM, H. H. & BARTER, P. J. 1991. Hepatic lipase promotes a loss of apolipoprotein AI from triglyceride-enriched human high density lipoproteins during incubation in vitro. *Arteriosclerosis, Thrombosis, and Vascular Biology,* 11, 415-422.

COLLABORATION, A. P. C. S. 2004. Body mass index and cardiovascular disease in the Asia-Pacific Region: an overview of 33 cohorts involving 310 000 participants. *International Journal of Epidemiology,* 33, 751-758.

COLLABORATION, E. R. F. 2009. Lipoprotein (a) concentration and the risk of coronary heart disease, stroke, and nonvascular mortality. *JAMA: the journal of the American Medical Association,* 302, 412.

COLLINS, R. G., VELJI, R., GUEVARA, N. V., HICKS, M. J., CHAN, L. & BEAUDET, A. L. 2000. P-Selectin or intercellular adhesion molecule (ICAM)-1 deficiency substantially protects against atherosclerosis in apolipoprotein E–deficient mice. *The Journal of experimental medicine,* 191, 189-194.

CROOK, M. A. 2012. *Clinical Biochemistry and Metabolic Medicine Eighth Edition*, CRC Press.

DAOUSI, C., CASSON, I., GILL, G., MACFARLANE, I., WILDING, J. & PINKNEY, J. 2006. Prevalence of obesity in type 2 diabetes in secondary care: association with cardiovascular risk factors. *Postgraduate medical journal,* 82, 280-284.

DEB, A. & CAPLICE, N. M. 2004. Lipoprotein (a): new insights into mechanisms of atherogenesis and thrombosis. *Clinical cardiology,* 27, 258-264.

DEFRONZO, R. A. & FERRANNINI, E. 1991. Insulin resistance: a multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. *Diabetes care,* 14, 173-194.

DETECTION, E. P. O. 2001. EVALUATION, AND TREATMENT OF HIGH BLOOD CHOLESTEROL IN ADULTS. Executive summary of the third report of The National Cholesterol Education Program (NCEP) Expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). *Jama,* 285, 2486-2497.

DIEPLINGER, H. & UTERMANN, G. 1999. The seventh myth of lipoprotein (a): where and how is it assembled? *Current opinion in lipidology,* 10, 275-284.

DIXON, J. L. & GINSBERG, H. N. Hepatic synthesis of lipoproteins and apolipoproteins. Seminars in liver disease, 1992. 364-372.

DONG, Z. M., CHAPMAN, S. M., BROWN, A. A., FRENETTE, P. S., HYNES, R. O. & WAGNER, D. D. 1998. The combined role of P-and E-selectins in atherosclerosis. *Journal of Clinical Investigation,* 102, 145.

DOUCET, C., HUBY, T., RUIZ, J., CHAPMAN, M. J. & THILLET, J. 1995. Non-enzymatic glycation of lipoprotein (a) in vitro and in vivo. *Atherosclerosis,* 118, 135-143.

DUBÉ, J. B., BOFFA, M. B., HEGELE, R. A. & KOSCHINSKY, M. L. 2012. Lipoprotein (a): more interesting than ever after 50 years. *Current opinion in lipidology,* 23, 133-140.

DUDEKHULA, B., ANWAR, N., LALU, J. & REDDY, K. 2012. Correlation between blood sugars and body maas index with blood pressure among the type 2 diabetic adults. *Asian J Exp. Biol. Sci,* 3, 378-383.

EDELSTEIN, C., PFAFFINGER, D., HINMAN, J., MILLER, E., LIPKIND, G., TSIMIKAS, S., BERGMARK, C., GETZ, G. S., WITZTUM, J. L. & SCANU, A. M. 2003. Lysine-phosphatidylcholine adducts in kringle V impart unique immunological and potential pro-inflammatory properties to human apolipoprotein (a). *Journal of Biological Chemistry,* 278, 52841-52847.

EL-GARI M, Ahmed Mohammed Ahmed; Hisham Ali Waggiallah and Mahmoud. 2013. Lipoprotein (a) level and other laboratory variables in diabetic patients.*Egyptian academic journal of biological sciences,* 5, 107-122.

EL-HAZMI, M. & WARSY, A. 1999. Obesity and overweight in Type II diabetes mellitus patients in Saudi Arabia. *Saudi medical journal,* 20, 167-172.

EPSTEIN, F. H. & ROSS, R. 1999. Atherosclerosis—an inflammatory disease. *New England journal of medicine,* 340, 115-126.

EREM, C., DEĞER, O., BOSTAN, M., OREM, A., SÖNMEZ, M., ULUSOY, S. & TELATAR, M. 1999. Plasma lipoprotein (a) concentrations in hypothyroid, euthyroid and hyperthyroid subjects. *Acta cardiologica,* 54, 77-81.

ETINGIN, O., HAJJAR, D., HAJJAR, K., HARPEL, P. & NACHMAN, R. 1991. Lipoprotein (a) regulates plasminogen activator inhibitor-1 expression in endothelial cells. A potential mechanism in thrombogenesis. *Journal of Biological Chemistry,* 266, 2459-2465.

EVANS, K., BURDGE, G. C., WOOTTON, S. A., CLARK, M. L. & FRAYN, K. N. 2002. Regulation of dietary fatty acid entrapment in subcutaneous adipose tissue and skeletal muscle. *Diabetes,* 51, 2684-2690.

FEBBRAIO, M., PODREZ, E. A., SMITH, J. D., HAJJAR, D. P., HAZEN, S. L., HOFF, H. F., SHARMA, K. & SILVERSTEIN, R. L. 2000. Targeted disruption of the class B scavenger receptor CD36 protects against atherosclerotic lesion development in mice. *Journal of Clinical Investigation,* 105, 1049-1056.

FEDERATION, I. D. 2011. IDF Diabetes Atlas. 5th edition ed. Brussels, Belgium: International Diabetes Federation.

FEDERATION, I. D. 2013. IDF Diabetes Atlas. 6th edition ed. Brussels, Belgium: International Diabetes Federation

FRANK, S., DUROVIC, S., KOSTNER, K. & KOSTNER, G. M. 1995. Inhibitors for the in vitro assembly of Lp (a). *Arteriosclerosis, thrombosis, and vascular biology,* 15, 1774-1780.

FRANK, S., KRASZNAI, K., DUROVIC, S., LOBENTANZ, E.-M., DIEPLINGER, H., WAGNER, E., ZATLOUKAL, K., COTTEN, M. & UTERMANN, G. 1994. High-level expression of various apolipoprotein (a) isoforms by" transferrinfection": the role of kringle IV sequences in the extracellular association with low-density lipoprotein. *Biochemistry,* 33, 12329-12339.

GAURAV, R., ARCHITA, S., MANISH, K., KARISHMA, J., ARUNDHATI, C., PRAVIR, K. & RASHMI, K. A. 2012. Clinical management of lipid profile, renal and liver function versus HbA1c profile in diabetes affected patients of Vellore, Tamil Nadu, India. *African Journal of Pharmacy and pharmacology,* 6, 2832-2836.

GEETHANJALI, F., LUTHRA, K., LINGENHEL, A., KANAGASABA-PATHY, A., JACOB, J., SRIVASTAVA, L. M., VASISHT, S., KRAFT, H.-G. & UTERMANN, G. 2003. Analysis of the apo (a) size

polymorphism in Asian Indian populations: association with Lp (a) concentration and coronary heart disease. *Atherosclerosis,* 169, 121-130.
GERICH, J. E. 1998. The genetic basis of type 2 diabetes mellitus: impaired insulin secretion versus impaired insulin sensitivity. *Endocrine reviews,* 19, 491-503.
GILL, G. 2004. *Unstable and brittle diabetes*, Taylor & Francis.
GINSBERG, H. N. & ILLINGWORTH, D. R. 2001. Postprandial dyslipidemia: an atherogenic disorder common in patients with diabetes mellitus. *The American journal of cardiology,* 88, 9-15.
GORDON, L., RAGOOBIRSINGH, D., ST ERROL, Y., CHOO-KANG, E., MCGROWDER, D. & MARTORELL, E. 2010. Lipid profile of type 2 diabetic and hypertensive patients in the Jamaican population. *Journal of laboratory physicians,* 2, 25.
GRAINGER, D. J., KEMP, P. R., LIU, A. C., LAWN, R. M. & METCALFE, J. C. 1994. Activation of transforming growth factor-β is inhibited in transgenic apolipoprotein (a) mice. *Nature*, 370, 460-462.
GRIES, A., NIMPF, J., NIMPF, M., WURM, H. & KOSTNER, G. M. 1987. Free and Apo B-associated Lpa-specific protein in human serum. *Clinica chimica acta,* 164, 93-100.
GROUP, U. P. D. S. 1995. UK Prospective Diabetes Study 16: overview of 6 years' therapy of type II diabetes: a progressive disease. *Diabetes,* 44, 1249-1258.
GUARIGUATA, L., WHITING, D., HAMBLETON, I., BEAGLEY, J., LINNENKAMP, U. & SHAW, J. 2014. Global estimates of diabetes prevalence for 2013 and projections for 2035. *Diabetes research and clinical practice,* 103, 137-149.
GURR, M. I., HARWOOD, J. L. & FRAYN, K. N. 2002. Lipid transport. *Lipid Biochemistry, 5th Edition*, 170-214.
HABIB, S. & ASLAM, M. 2004. Lipids and lipoprotein (a) concentrations in Pakistani patients with type 2 diabetes mellitus. *Diabetes, Obesity and Metabolism,* 6, 338-343.
HABIB, S. S. 2013. Serum lipoprotein (a) and high sensitivity C reactive protein levels in Saudi patients with type 2 diabetes mellitus and their relationship with glycemic control. *Turkish Journal of Medical Sciences,* 43, 333-338.
HABIB, S. S. & ASLAM, M. 2003. High risk levels of lipoprotein (a) in Pakistani patients with type 2 diabetes mellitus. *Saudi medical journal,* 24, 647-651.
HABIB, S. S., ASLAM, M., SHAH, S. F. & NAVEED, A. K. 2009. Lipoprotein (a) is associated with basal insulin levels in patients with type 2 Diabetes Mellitus. *Arq Bras Cardiol,* 93, 28-33.
HACHEM, S. B. & MOORADIAN, A. D. 2006. Familial Dyslipidaemias. *Drugs,* 66, 1949-1969.
HAJJAR, K. A., GAVISHI, D., BRESLOW, J. L. & NACHMAN, R. L. 1989. Lipoprotein (a) modulation of endothelial cell surface fibrinolysis and its potential role in atherosclerosis. *Nature*, 339, 303-305.
HASSING, H., SURENDRAN, R., MOOIJ, H., STROES, E., NIEUWDORP, M. & DALLINGA-THIE, G. 2012. Pathophysiology of hypertriglyceridemia. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids,* 1821, 826-832.
HEGELE, R. A. 1999. Paraoxonase genes and disease. *Annals of medicine,* 31, 217-224.
HELLER, F. R., JAMART, J., HONORE, P., DERUE, G., NOVIK, V., GALANTI, L., PARFONRY, A., HONDEKIJN, J.-C. & BUYSSCHAERT, M. 1993. Serum lipoprotein (a) in patients with diabetes mellitus. *Diabetes Care,* 16, 819-823.
HELLER, S. 2004. Weight gain during insulin therapy in patients with type 2 diabetes mellitus. *Diabetes research and clinical practice,* 65, S23-S27.
HERNÁNDEZ, C., CHACÓN, P., GARCÍA-PASCUAL, L. & SIMÓ, R. 2001. Differential influence of LDL cholesterol and triglycerides on lipoprotein (a) concentrations in diabetic patients. *Diabetes Care,* 24, 350-355.

HIMABINDU, Y., SRIHARIBABU, M., ALEKHYA, K., SAISUMANTH, K., LAKSHMANRAO, N. & KOMALI, K. 2013. Correlations between anthropometry and lipid profile in type 2 diabetics. *Indian journal of endocrinology and metabolism,* 17, 727.

HOFMANN, M. A., DRURY, S., FU, C., QU, W., TAGUCHI, A., LU, Y., AVILA, C., KAMBHAM, N., BIERHAUS, A. & NAWROTH, P. 1999. RAGE mediates a novel proinflammatory axis: a central cell surface receptor for S100/calgranulin polypeptides. *Cell,* 97, 889-901.

HOLT, R. I., COCKRAM, C., FLYVBJERG, A. & GOLDSTEIN, B. J. 2011. *Textbook of diabetes*, John Wiley & Sons.

ICHINOSE, A. 2008. [Hyperlipoprotein (a)-emia determined by genetic polymorphisms in apolipoprotein (a) gene]. *Brain and nerve= Shinkei kenkyu no shinpo,* 60, 1307-1317.

INDUMATI, V., PATIL, V. S., KRISHNASWAMY, D., SATISHKUMAR, D., VIJAY, V., MAHESH, S. & RAJESHWARI, V. 2011. NON-HDL CHOLESTEROL AND LDL-C/HDL-C RATIO IN TYPE II DIABETIC PATIENTS. *International Journal of Pharma & Bio Sciences,* 2.

IVANDIC, B., CASTELLANI, L. W., WANG, X.-P., QIAO, J.-H., MEHRABIAN, M., NAVAB, M., FOGELMAN, A. M., GRASS, D. S., SWANSON, M. E. & DE BEER, M. C. 1999. Role of group II secretory phospholipase A2 in atherosclerosis 1. Increased atherogenesis and altered lipoproteins in transgenic mice expressing group IIa phospholipase A2. *Arteriosclerosis, thrombosis, and vascular biology,* 19, 1284-1290.

JENNER, J. L., SEMAN, L. J., MILLAR, J. S., LAMON-FAVA, S., WELTY, F. K., DOLNIKOWSKI, G. G., MARCOVINA, S. M., LICHTENSTEIN, A. H., BARRETT, P. H. R. & DELUCA, C. 2005. The metabolism of apolipoproteins (a) and B-100 within plasma lipoprotein (a) in human beings. *Metabolism,* 54, 361-369.

KADIKI, O. & ROAED, R. 1999. Epidemiological and clinical patterns of diabetes mellitus in Benghazi, Libyan Arab Jamahiriya. *Eastern Mediterranean health journal= La revue de sante de la Mediterranee orientale= al-Majallah al-sihhiyah li-sharq al-mutawassit,* 5, 6-13.

KAGAWA, A., AZUMA, H., AKAIKE, M., KANAGAWA, Y. & MATSUMOTO, T. 1999. Aspirin reduces apolipoprotein (a)(apo (a)) production in human hepatocytes by suppression of apo (a) gene transcription. *Journal of Biological Chemistry,* 274, 34111-34115.

KAHN, S. E. 2001. The importance of β-cell failure in the development and progression of type 2 diabetes. *The Journal of Clinical Endocrinology & Metabolism,* 86, 4047-4058.

KAMSTRUP, P. R., TYBJÆRG-HANSEN, A., STEFFENSEN, R. & NORDESTGAARD, B. G. 2008. Pentanucleotide repeat polymorphism, lipoprotein (a) levels, and risk of ischemic heart disease. *The Journal of Clinical Endocrinology & Metabolism,* 93, 3769-3776.

KANDULA, R. & SHEGOKAR, V. E. 2013. a study of lipid profile in patients with type-2 diabetes mellitus. *MRIMS journal of health sciences,* 1, 23-26.

KANNEL, W. 2005. Risk stratification of dyslipidemia: insights from the Framingham Study. *Current Medicinal Chemistry-Cardiovascular & Hematological Agents,* 3, 187-193.

KARPE, F., DICKMANN, J. R. & FRAYN, K. N. 2011. Fatty acids, obesity, and insulin resistance: time for a reevaluation. *Diabetes,* 60, 2441-2449.

KHAN, H., SOBKI, S. & KHAN, S. 2007. Association between glycaemic control and serum lipids profile in type 2 diabetic patients: HbA1c predicts dyslipidaemia. *Clinical and experimental medicine,* 7, 24-29.

KISSEBAH, A. H. & KRAKOWER, G. R. 1994. Regional adiposity and morbidity. *Physiological Reviews,* 74, 761-811.

KLAYA, F., DURLACH, V., BERTIN, E., MONIER, F., MONBOISSE, J.-C. & GILLERY, P. 1997. Evaluation of serum glycated lipoprotein (a) levels in noninsulin-dependent diabetic patients. *Clinical biochemistry,* 30, 227-230.

KNOWLES, J. W., REDDICK, R. L., JENNETTE, J. C., SHESELY, E. G., SMITHIES, O. & MAEDA, N. 2000. Enhanced atherosclerosis and kidney dysfunction in eNOS–/–Apoe–/–mice are ameliorated by enalapril treatment. *The Journal of clinical investigation,* 105, 451-458.

KO, S. H., SONG, K.-H., AHN, Y.-B., YOO, S.-J., SON, H.-S., YOON, K.-H., CHA, B.-Y., LEE, K.-W., SON, H.-Y. & KANG, S.-K. 2003. The effect of rosiglitazone on serum lipoprotein (a) levels in Korean patients with type 2 diabetes mellitus. *Metabolism,* 52, 731-734.

KOHEI, K. 2010. Pathophysiology of type 2 diabetes and its treatment policy. *Jpn Med Assoc,* 53, 41-46.

KOLLERITS, B., AUINGER, M., REISIG, V., KÄSTENBAUER, T., LINGENHEL, A., IRSIGLER, K., PRAGER, R. & KRONENBERG, F. 2006. Lipoprotein (a) as a predictor of cardiovascular disease in a prospectively followed cohort of patients with type 1 diabetes. *Diabetes care,* 29, 1661-1663.

KOLOVOU, G. D., ANAGNOSTOPOULOU, K. K., SALPEA, K. D., HOURSALAS, I. S., PETROPOULOS, I., BILIANOU, H. I., DAMASKOS, D. S., GIANNAKOPOULOU, V. N. & COKKINOS, D. V. 2006. Influence of triglycerides on other plasma lipids in middle-aged men intended for hypolipidaemic treatment. *Hellenic J Cardiol,* 47, 78-83.

KOSTNER, K., CLODI, M., BODLAJ, G., WATSCHINGER, B., HORL, W., DERFLER, K. & HUBER, K. 1998a. Decreased urinary apolipoprotein (a) excretion in patients with impaired renal function. *European journal of clinical investigation,* 28, 447-452.

KOSTNER, K. M., BANYAI, S., BANYAI, M., BODLAJ, G., MAURER, G., DERFLER, K., HÖRL, W. H. & OBERBAUER, R. 1998b. Urinary apolipoprotein (a) excretion in patients with proteinuria. *Annals of medicine,* 30, 497-502.

KOSTNER, K. M. & KOSTNER, G. M. 2002. Lipoprotein (a): still an enigma? *Current opinion in lipidology,* 13, 391-396.

KOSTNER, K. M., MÄRZ, W. & KOSTNER, G. M. 2013. When should we measure lipoprotein (a)? *European heart journal,* 34, 3268-3276.

KOSTNER, K. M., MAURER, G., HUBER, K., STEFENELLI, T., DIEPLINGER, H., STEYRER, E. & KOSTNER, G. M. 1996. Urinary Excretion of Apo (a) Fragments Role in Apo (a) Catabolism. *Arteriosclerosis, thrombosis, and vascular biology,* 16, 905-911.

KRAUSS, M. & RONALD, M. 1998. Atherogenicity of triglyceride-rich lipoproteins. *The American journal of cardiology,* 81, 13B-17B.

KRAUSS, R. M. 1998. Triglycerides and atherogenic lipoproteins: rationale for lipid management. *The American journal of medicine,* 105, 58S-62S.

KRENTZ, A. J. 2003. Lipoprotein abnormalities and their consequences for patients with type 2 diabetes. *Diabetes, Obesity and Metabolism,* 5, s19-s27.

KRONENBERG, F., IKEWAKI, K., SCHAEFER, J. R., KÖNIG, P. & DIEPLINGER, H. Kinetic studies of atherogenic lipoproteins in hemodialysis patients: do they tell us more about their pathology? Seminars in dialysis, 2006. 554-560.

KRONENBERG, F., TRENKWALDER, E., LINGENHEL, A., FRIEDRICH, G., LHOTTA, K., SCHOBER, M., MOES, N., KÖNIG, P., UTERMANN, G. & DIEPLINGER, H. 1997. Renovascular arteriovenous differences in Lp [a] plasma concentrations suggest removal of Lp [a] from the renal circulation. *Journal of lipid research,* 38, 1755-1763.

LAMBERT, J. E. & PARKS, E. J. 2012. Postprandial metabolism of meal triglyceride in humans. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids,* 1821, 721-726.

LANKTREE, M. B., ANAND, S. S., YUSUF, S. & HEGELE, R. A. 2010. Comprehensive analysis of genomic variation in the LPA locus and its relationship to plasma lipoprotein (a) in South Asians, Chinese, and European Caucasians. *Circulation: Cardiovascular Genetics,* 3, 39-46.

LAWN, R. M., BOONMARK, N. W., SCHWARTZ, K., LINDAHL, G. E., WADE, D. P., BYRNE, C. D., FONG, K. J., MEER, K. & PATTHY, L. 1995. The recurring evolution of lipoprotein (a) Insights from cloning of hedgehog apolipoprotein (a). *Journal of Biological Chemistry,* 270, 24004-24009.

LAWN, R. M., WADE, D. P., HAMMER, R. E., CHIESA, G., VERSTUYFT, J. G. & RUBIN, E. M. 1992. Atherogenesis in transgenic mice expressing human apolipoprotein (a). *Nature,* 360, 670-672.

LEE, R. T. & LIBBY, P. 1997. The unstable atheroma. *Arteriosclerosis, thrombosis, and vascular biology,* 17, 1859-1867.

LEE, W. L., CHEUNG, A. M., CAPE, D. & ZINMAN, B. 2000. Impact of diabetes on coronary artery disease in women and men: a meta-analysis of prospective studies. *Diabetes care,* 23, 962-968.

LEEPER, N. J. & HEIDENREICH, P. A. 2008. Clinical significance of high-density lipoprotein cholesterol in patients with low low-density lipoprotein cholesterol. *Journal of the American College of Cardiology,* 51, 49-55.

LI, Y., LUKE, M. M., SHIFFMAN, D. & DEVLIN, J. J. 2011. Genetic variants in the apolipoprotein (a) gene and coronary heart disease. *Circulation: Cardiovascular Genetics,* 4, 565-573.

LIBBY, P. 2000. Changing concepts of atherogenesis. *Journal of internal medicine,* 247, 349-358.

LLOYD-JONES, D. M., LEIP, E. P., LARSON, M. G., D'AGOSTINO, R. B., BEISER, A., WILSON, P. W., WOLF, P. A. & LEVY, D. 2006. Prediction of lifetime risk for cardiovascular disease by risk factor burden at 50 years of age. *Circulation,* 113, 791-798.

LOSCALZO, J., WEINFELD, M., FLESS, G. M. & SCANU, A. M. 1990. Lipoprotein (a), fibrin binding, and plasminogen activation. *Arteriosclerosis, Thrombosis, and Vascular Biology,* 10, 240-245.

LUNDSTAM, U., HURT-CAMEJO, E., OLSSON, G., SARTIPY, P., CAMEJO, G. & WIKLUND, O. 1999. Proteoglycans contribution to association of Lp (a) and LDL with smooth muscle cell extracellular matrix. *Arteriosclerosis, thrombosis, and vascular biology,* 19, 1162-1167.

LUSIS, A. J. 2000. Atherosclerosis. *Nature,* 407, 233-41.

MACA, T., MLEKUSCH, W., DOWEIK, L., BUDINSKY, A., BISCHOF, M., MINAR, E. & SCHILLINGER, M. 2007. Influence and interaction of diabetes and lipoprotein (a) serum levels on mortality of patients with peripheral artery disease. *European journal of clinical investigation,* 37, 180-186.

MAGGIO, C. A. & PI-SUNYER, F. X. 2003. Obesity and type 2 diabetes. *Endocrinology and metabolism clinics of North America,* 32, 805-822.

MAJEED, A., EL-SAYED, A. A., KHOJA, T., ALSHAMSAN, R., MILLETT, C. & RAWAF, S. 2014. Diabetes in the Middle-East and North Africa: An update. *Diabetes research and clinical practice,* 103, 218-222.

MAKI, K. C., KRITSCH, K., FOLEY, S., SONERU, I. & DAVIDSON, M. H. 1997. Age-dependence of the relationship between adiposity and serum low density lipoprotein cholesterol in men. *Journal of the American College of Nutrition,* 16, 578-583.

MALAGUARNERA, M., VACANTE, M., RUSSO, C., MALAGUARNERA, G., ANTIC, T., MALAGUARNERA, L., BELLA, R., PENNISI, G., GALVANO, F. & FRIGIOLA, A. 2012. Lipoprotein (a) in cardiovascular diseases. *BioMed research international,* 2013.

MALMSTRÖM, R., PACKARD, C., CASLAKE, M., BEDFORD, D., STEWART, P., YKI-JÄRVINEN, H., SHEPHERD, J. & TASKINEN, M.-R. 1997. Defective regulation of triglyceride metabolism by insulin in the liver in NIDDM. *Diabetologia,* 40, 454-462.

MARATHE, S., KURIAKOSE, G., WILLIAMS, K. J. & TABAS, I. 1999. Sphingomyelinase, an enzyme implicated in atherogenesis, is present in atherosclerotic lesions and binds to specific

components of the subendothelial extracellular matrix. *Arteriosclerosis, thrombosis, and vascular biology,* 19, 2648-2658.
MARCOVINA, S. M. & KOSCHINSKY, M. L. 1999. Lipoprotein (a) concentration and apolipoprotein (a) size A synergistic role in advanced atherosclerosis? *Circulation,* 100, 1151-1153.
MARCOVINA, S. M. & KOSCHINSKY, M. L. 2003. Evaluation of lipoprotein (a) as a prothrombotic factor: progress from bench to bedside. *Current opinion in lipidology,* 14, 361-366.
MARCOVINA, S. M., KOSCHINSKY, M. L., ALBERS, J. J. & SKARLATOS, S. 2003. Report of the National Heart, Lung, and Blood Institute Workshop on Lipoprotein (a) and Cardiovascular Disease: recent advances and future directions. *Clinical chemistry,* 49, 1785-1796.
MATSUMOTO, K., MIYAKE, S., YANO, M., UEKI, Y. & TOMINAGA, Y. 1997. Increase of lipoprotein (a) with troglitazone. *The Lancet,* 350, 1748-1749.
MATSUMOTO, M., HAN, S., KITAMURA, T. & ACCILI, D. 2006. Dual role of transcription factor FoxO1 in controlling hepatic insulin sensitivity and lipid metabolism. *The Journal of clinical investigation,* 116, 2464-2472.
MCCORMICK, S. P. 2004. Lipoprotein (a): biology and clinical importance. *The Clinical Biochemist Reviews,* 25, 69.
MCLEAN, J. W., TOMLINSON, J. E., KUANG, W.-J., EATON, D. L., CHEN, E. Y., FLESS, G. M., SCANU, A. M. & LAWN, R. M. 1987. cDNA sequence of human apolipoprotein (a) is homologous to plasminogen. *Nature,* 330, 132-137.
MENGESHA, A. Y. 2008. Hypertension and related risk factors in type 2 diabetes mellitus (DM) patients in Gaborone City Council (GCC) clinics, Gaborone, Botswana. *African health sciences,* 7.
MINNICH, A., NORDESTGAARD, B. & ZILVERSMIT, D. 1989. A novel explanation for the reduced LDL cholesterol in severe hypertriglyceridemia. *Journal of lipid research,* 30, 347-355.
MOHAMMED, A. H., MODAWE, G. A., ELSONNI, B. & ELTOM, K. 2012. Biochemical Assessment in Sudanese Patients with Type 2 Diabetes Mellitus. *SUDANESE JOURNAL OF PUBLIC HEALTH,* 7, 123-125.
MOORADIAN, A. D., HAAS, M. J., WEHMEIER, K. R. & WONG, N. C. 2008. Obesity-related Changes in High-density Lipoprotein Metabolism. *Obesity,* 16, 1152-1160.
MORA, S., KAMSTRUP, P. R., RIFAI, N., NORDESTGAARD, B. G., BURING, J. E. & RIDKER, P. M. 2010. Lipoprotein (a) and risk of type 2 diabetes. *Clinical chemistry,* 56, 1252-1260.
MURRAY, K., RODWELL, V., BENDER, D., BOTHAM, K. M., WEIL, P. A. & KENNELLY, P. J. 2009. *Harper's Illustrated Biochemistry. 28*, New York: McGraw-Hill.
NARUSZEWICZ, M., SELINGER, E., DUFOUR, R. & DAVIGNON, J. 1992. Probucol protects lipoprotein (a) against oxidative modification. *Metabolism,* 41, 1225-1228.
NASRI, H. 2006. THE INFLUENCE OF SERUM LDL-CHOLESTEROL AND HDL-CHOLESTEROL ON SYSTOLIC BLOOD PRESSURE OF TYPE II DIABETIC PATIENTS WITH VARIOUS KIDNEYS FUNCTION NOT YET ON DIALYSIS. *Pak J Physiol,* 2.
NASRI, H., BEHRADMANESH, S., AHMADI, A., BARADARAN, A., NASRI, P. & RAFIEIAN-KOPAEI, M. 2014. Association of serum lipids with level of blood pressure in type 2 diabetic patients. *J Renal Inj Prev,* 3, 43-46.
NAVAB, M., HAMA, S. Y., READY, S. T., NG, C. J., VAN LENTEN, B. J., LAKS, H. & FOGELMAN, A. M. 2002. Oxidized lipids as mediators of coronary heart disease. *Current opinion in lipidology,* 13, 363-372.
NEELE, D., DE WIT, E. & PRINCEN, H. 1999. Insulin suppresses apolipoprotein (a) synthesis by primary cultures of cynomolgus monkey hepatocytes. *Diabetologia,* 42, 41-44.

NIELSEN, L. B. 1999. Atherogenecity of lipoprotein (a) and oxidized low density lipoprotein: insight from in vivo studies of arterial wall influx, degradation and efflux. *Atherosclerosis,* 143, 229-243.

NISHIMURA, H. 2012. [Hypertension and abnormal lipid metabolism]. *Nihon Rinsho,* 70 Suppl 5, 258-62.

NOGUEIRA, A., MARCOPITO, L., DEMEDICINA, E., PAULO, S., LANAS, F., DELAFRONTERA, U., GALDAMES, D., WANG, J., FANG, J. & HU, F. 1996. Body mass index and cardiovascular disease risk factors in seven Asian and five Latin American centers: Data from the International Clinical Epidemiology Network (INCLEN). *OBESITY RESEARCH,* 4, 221-228.

NORDESTGAARD, B. G., CHAPMAN, M. J., RAY, K., BORÉN, J., ANDREOTTI, F., WATTS, G. F., GINSBERG, H., AMARENCO, P., CATAPANO, A. & DESCAMPS, O. S. 2010. Lipoprotein (a) as a cardiovascular risk factor: current status. *European heart journal,* 31, 2844-2853.

OGBERA, A. O. & AZENABOR, A. O. 2010. Lipoprotein (a), C-reactive protein and some metabolic cardiovascular risk factors in type 2 DM. *Diabetol Metab Syndr,* 2, 51.

OGORELKOVA, M., KRAFT, H. G., EHNHOLM, C. & UTERMANN, G. 2001. Single nucleotide polymorphisms in exons of the apo (a) kringles IV types 6 to 10 domain affect Lp (a) plasma concentrations and have different patterns in Africans and Caucasians. *Human molecular genetics,* 10, 815-824.

OVALLE, F. & BELL, D. 1999. Troglitazone's effect on lipoprotein (a) levels. *Diabetes care,* 22, 859-860.

PACKARD, C. J., FORD, I., ROBERTSON, M., SHEPHERD, J., BLAUW, G. J., MURPHY, M. B., BOLLEN, E. L., BUCKLEY, B. M., COBBE, S. M. & GAW, A. 2005. Plasma lipoproteins and apolipoproteins as predictors of cardiovascular risk and treatment benefit in the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER). *Circulation,* 112, 3058-3065.

PANAGOTOPULOS, S. E., HORACE, E. M., MAIORANO, J. N. & DAVIDSON, W. S. 2001. Apolipoprotein AI adopts a belt-like orientation in reconstituted high density lipoproteins. *Journal of Biological Chemistry,* 276, 42965-42970.

PANAROTTO, D., REMILLARD, P., BOUFFARD, L. & MAHEUX, P. 2002. Insulin resistance affects the regulation of lipoprotein lipase in the postprandial period and in an adipose tissue-specific manner. *European journal of clinical investigation,* 32, 84-92.

PATIDAR, D. & DWIVEDI, S. K. 2012. Diabetes Mellitus: An Update. *International Journal of Pharmaceutical & Research sciences,* 1, 259-276.

PATIL, M., KUMAR, N., NUSRATH, A. & JAYARAM, S. 2014. ASSOCIATION OF HbA1c WITH SERUM LIPID PROFILE AND LIPOPROTEIN (a) IN TYPE 2 DIABETES MELLITUS. *International Journal of Current Research and Review,* 6, 20-25.

PIRILLO, A., NORATA, G. D. & CATAPANO, A. L. 2013. High-density lipoprotein subfractions-what the clinicians need to know. *Cardiology,* 124, 116-125.

PODREZ, E. A., FEBBRAIO, M., SHEIBANI, N., SCHMITT, D., SILVERSTEIN, R. L., HAJJAR, D. P., COHEN, P. A., FRAZIER, W. A., HOFF, H. F. & HAZEN, S. L. 2000. Macrophage scavenger receptor CD36 is the major receptor for LDL modified by monocyte-generated reactive nitrogen species. *The Journal of clinical investigation,* 105, 1095-1108.

POORSOLTAN, N., AHMADI, R., FOROUTAN, M. & KHOSRAVY, S. 2013. Association between Hyperglycemia and Lipid Profile in Prediabetic and Diabetic Patients. *4th International Conference on Medical, Biological and Pharmaceutical Sciences.* Dubai (UAE).

QI, Q. & QI, L. 2012. Lipoprotein (a) and cardiovascular disease in diabetic patients. *Clinical lipidology,* 7, 397-407.

RADER, D. J. 2012. High-density lipoprotein particle number: a better measure to quantify high-density lipoprotein? *Journal of the American College of Cardiology,* 60, 517-520.
RAINWATER, D. L. & HAFFNER, S. M. 1998. Insulin and 2-hour glucose levels are inversely related to Lp (a) concentrations controlled for LPA genotype. *Arteriosclerosis, thrombosis, and vascular biology,* 18, 1335-1341.
RAINWATER, D. L., MACCLUER, J. W., STERN, M. P., VANDEBERG, J. L. & HAFFNER, S. M. 1994. Effects of NIDDM on lipoprotein (a) concentration and apolipoprotein (a) size. *Diabetes,* 43, 942-946.
RAMHARACK, R., BARKALOW, D. & SPAHR, M. A. 1998. Dominant negative effect of TGF-β1 and TNF-α on basal and IL-6–induced lipoprotein (a) and apolipoprotein (a) mRNA expression in primary monkey hepatocyte cultures. *Arteriosclerosis, thrombosis, and vascular biology,* 18, 984-990.
RAMONA, G., IOAN, C., SIMONA, T., LUMINITA, P. & SIMONA GH, L. M. 2011. Relationship between glycosylated hemoglobin and lipid metabolism in patients with type 2 diabetes. *StudiaUniversitatis "Vasile Goldiş", SeriaŞtiinţeleVieţii,* 21, 313-8.
RAO, R., ALBERS, J. J., WOLFBAUER, G. & POWNALL, H. J. 1997. Molecular and macromolecular specificity of human plasma phospholipid transfer protein. *Biochemistry,* 36, 3645-3653.
REASNER, C. & DEFRONZO, R. 2006. Classification and Diagnosis of Diabetes Mellitus. May.
REMALEY, A. T., STONIK, J. A., DEMOSKY, S. J., NEUFELD, E. B., BOCHAROV, A. V., VISHNYAKOVA, T. G., EGGERMAN, T. L., PATTERSON, A. P., DUVERGER, N. J. & SANTAMARINA-FOJO, S. 2001. Apolipoprotein specificity for lipid efflux by the human ABCAI transporter. *Biochemical and biophysical research communications,* 280, 818-823.
RIBAULT, A., DUROU, M., LETELLIER, C., WOJCIK, F., POIRIER, J. & RUELLAND, A. 2000. Determination of lipoprotein (a) concentrations and apolipoprotein (a) molecular weights in diabetic patients. *Diabetes & metabolism,* 26, 107-112.
ROAEID, R. & KABLAN, A. 2010. Diabetes mortality and causes of death in Benghazi: a 5-year retrospective analysis of death certificates. *EMHJ,* 16, 65-69.
ROAEID, R. & KADIKI, O. 2011. Prevalence of long-term complications among Type 2 diabetic patients in Benghazi, Libya. *Journal of Diabetology,* 3, 5.
ROCHE DIAGNOSTICS. 2007. Method manual of COBAS INTEGRA 400/ 700/ 800.
ROCHE DIAGNOSTICS. 2009. Method manual of COBAS e 411 analyzer.
ROHLFING, C. L., WIEDMEYER, H.-M., LITTLE, R. R., ENGLAND, J. D., TENNILL, A. & GOLDSTEIN, D. E. 2002. Defining the relationship between plasma glucose and HbA1c analysis of glucose profiles and HbA1c in the Diabetes Control and Complications Trial. *Diabetes care,* 25, 275-278.
ROSEDIANI, M., AZIDAH, A. K. & MAFAUZY, M. 2006. Correlation between fasting plasma glucose, post prandial glucose and glycated haemoglobin and fructosamine. *Med J Malaysia,* 61, 67-71.
RYE, K.-A. & BARTER, P. J. 2004. Formation and metabolism of prebeta-migrating, lipid-poor apolipoprotein AI. *Arteriosclerosis, thrombosis, and vascular biology,* 24, 421-428.
SANDHU, H. S., KOLEY, S. & SANDHU, K. S. 2008. A study of correlation between lipid profile and body mass index (BMI) in patients with diabetes mellitus. *J Hum Ecol,* 24, 227-229.
SCHEEN, A. J. 2003. Current management strategies for coexisting diabetes mellitus and obesity. *Drugs,* 63, 1165-1184.
SCHÖNBECK, U., MACH, F., SUKHOVA, G. K., HERMAN, M., GRABER, P., KEHRY, M. R. & LIBBY, P. 2000a. CD40 ligation induces tissue factor expression in human vascular smooth muscle cells. *The American journal of pathology,* 156, 7-14.

SCHÖNBECK, U., SUKHOVA, G., SHIMIZU, K., MACH, F. & LIBBY, P. 2000b. Inhibition of CD40 signaling limits evolution of established atherosclerosis in mice. *Proceedings of the National Academy of Sciences,* 97, 7458-7463.

SCHULTZ, O., OBERHAUSER, F., SAECH, J., RUBBERT-ROTH, A., HAHN, M., KRONE, W. & LAUDES, M. 2010. Effects of inhibition of interleukin-6 signalling on insulin sensitivity and lipoprotein (a) levels in human subjects with rheumatoid diseases. *PloS one,* 5, e14328.

SHEIKHPOUR, R., SADEGHIAN, F. & RAJABI, S. 2013. Correlation between Glycated Hemoglobin, Serum Glucose and Serum Lipid Levels in Type 2 Diabetes. *Iranian Journal of Diabetes & Obesity (IJDO),* 5.

SHIH, D. M., XIA, Y.-R., WANG, X.-P., MILLER, E., CASTELLANI, L. W., SUBBANAGOUNDER, G., CHEROUTRE, H., FAULL, K. F., BERLINER, J. A. & WITZTUM, J. L. 2000. Combined serum paraoxonase knockout/apolipoprotein E knockout mice exhibit increased lipoprotein oxidation and atherosclerosis. *Journal of Biological Chemistry,* 275, 17527-17535.

SHOFF, S. M., KLEIN, R., MOSS, S. E., KLEIN, B. E. & CRUICKSHANKS, K. J. 1998. Weight change and glycemic control in a population-based sample of adults with older-onset diabetes. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences,* 53, M27-M32.

SINGLA, S., KAUR, K., KAUR, G., KAUR, H., KAUR, J. & JASWAL, S. 2009. Lipoprotein (a) in type 2 diabetes mellitus: Relation to LDL: HDL ratio and glycemic control. *International journal of diabetes in developing countries,* 29, 80.

SKÅLÉN, K., GUSTAFSSON, M., RYDBERG, E. K., HULTÉN, L. M., WIKLUND, O., INNERARITY, T. L. & BORÉN, J. 2002. Subendothelial retention of atherogenic lipoproteins in early atherosclerosis. *Nature,* 417, 750-754.

SMAOUI, M., HAMMAMI, S., CHAABA, R., ATTIA, N., HAMDA, K. B., MASMOUDI, A., MAHJOUB, S., BOUSSLAMA, A., FARHAT, M. B. & HAMMAMI, M. 2004. Lipids and lipoprotein (a) concentrations in Tunisian type 2 diabetic patients: relationship to glycemic control and coronary heart disease. *Journal of Diabetes and its Complications,* 18, 258-263.

SMITH, J. D., TROGAN, E., GINSBERG, M., GRIGAUX, C., TIAN, J. & MIYATA, M. 1995. Decreased atherosclerosis in mice deficient in both macrophage colony-stimulating factor (op) and apolipoprotein E. *Proceedings of the National Academy of Sciences,* 92, 8264-8268.

SMITH JR, S. C. 2007. Multiple risk factors for cardiovascular disease and diabetes mellitus. *The American journal of medicine,* 120, S3-S11.

SONE, H., YOSHIMURA, Y., TANAKA, S., IIMURO, S., OHASHI, Y., ITO, H., SEINO, H., ISHIBASHI, S., AKANUMA, Y. & YAMADA, N. 2007. Cross-sectional association between BMI, glycemic control and energy intake in Japanese patients with type 2 diabetes: analysis from the Japan Diabetes Complications Study. *Diabetes research and clinical practice,* 77, S23-S29.

SORAN, H. & DURRINGTON, P. N. 2011. Susceptibility of LDL and its subfractions to glycation. *Current opinion in lipidology,* 22, 254-261.

SPRAFKA, J. M., BURKE, G. L., FOLSOM, A. R., MCGOVERN, P. G. & HAHN, L. P. 1991. Trends in prevalence of diabetes mellitus in patients with myocardial infarction and effect of diabetes on survival: the Minnesota Heart Survey. *Diabetes Care,* 14, 537-543.

SRISAWASDI, P., CHALOEYSUP, S., TEERAJETGUL, Y., POCATHIKORN, A., SUKASEM, C., VANAVANAN, S. & KROLL, M. H. 2011. Estimation of plasma small dense LDL cholesterol from classic lipid measures. *American journal of clinical pathology,* 136, 20-29.

STAMLER, J., VACCARO, O., NEATON, J. D. & WENTWORTH, D. 1993. Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. *Diabetes care,* 16, 434-444.

STUMVOLL, M., GOLDSTEIN, B. J. & VAN HAEFTEN, T. W. 2005. Type 2 diabetes: principles of pathogenesis and therapy. *The Lancet,* 365, 1333-1346.
STUMVOLL, M., GOLDSTEIN, B. J. & VAN HAEFTEN, T. W. 2007. Pathogenesis of type 2 diabetes. *Endocrine research,* 32, 19-37.
SUZUKI, H., KURIHARA, Y., TAKEYA, M., KAMADA, N., KATAOKA, M., JISHAGE, K., UEDA, O., SAKAGUCHI, H., HIGASHI, T. & SUZUKI, T. 1997. A role for macrophage scavenger receptors in atherosclerosis and susceptibility to infection.
TACK, C., SMITS, P., DEMACKER, P. & STALENHOEF, A. 1999. Effect of troglitazone on lipoprotein (a) levels in obese subjects. *Diabetes care,* 22, 1752-1753.
TAN, K. 2009. Reverse cholesterol transport in type 2 diabetes mellitus. *Diabetes, obesity and metabolism,* 11, 534-543.
TASKINEN, M.-R. 2003. Diabetic dyslipidaemia: from basic research to clinical practice*. *Diabetologia,* 46, 733-749.
TESFAYE, F., NAWI, N., VAN MINH, H., BYASS, P., BERHANE, Y., BONITA, R. & WALL, S. 2006. Association between body mass index and blood pressure across three populations in Africa and Asia. *Journal of human hypertension,* 21, 28-37.
TSENG, C.-H. 2007. Body mass index and blood pressure in adult type 2 diabetic patients in Taiwan. *Circulation journal: official journal of the Japanese Circulation Society,* 71, 1749-1754.
TURNER, R., HOLMAN, R., CULL, C., STRATTON, I., MATTHEWS, D., FRIGHI, V., MANLEY, S., NEIL, A., MCELROY, K. & WRIGHT, D. 1998a. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Iancet,* 352, 837-853.
TURNER, R., MILLNS, H., NEIL, H., STRATTON, I., MANLEY, S., MATTHEWS, D. & HOLMAN, R. 1998b. Risk factors for coronary artery disease in non-insulin dependent diabetes mellitus: United Kingdom Prospective Diabetes Study (UKPDS: 23). *Bmj,* 316, 823-828.
TZIOMALOS, K., ATHYROS, V. G., WIERZBICKI, A. S. & MIKHAILIDIS, D. P. 2009. Lipoprotein a: where are we now? *Current opinion in cardiology,* 24, 351-357.
UTERMANN, G., MENZEL, H., KRAFT, H., DUBA, H., KEMMLER, H. & SEITZ, C. 1987. Lp (a) glycoprotein phenotypes. Inheritance and relation to Lp (a)-lipoprotein concentrations in plasma. *Journal of Clinical Investigation,* 80, 458.
VALENTI, K., AVEYNIER, E., LEAUTÉ, S., LAPORTE, F. & HADJIAN, A. J. 1999. Contribution of apolipoprotein (a) size, pentanucleotide TTTTA repeat and C/T (+ 93) polymorphisms of the apo (a) gene to regulation of lipoprotein (a) plasma levels in a population of young European Caucasians. *Atherosclerosis,* 147, 17-24.
VAN DER LINDE, N. A., SIJBRANDS, E. J., BOOMSMA, F. & VAN DEN MEIRACKER, A. H. 2006. Effect of Low-Density Lipoprotein Cholesterol on Angiotensin II Sensitivity A Randomized Trial With Fluvastatin. *Hypertension,* 47, 1125-1130.
VANCE, J. E. & VANCE, D. E. 2008. *Biochemistry of lipids, lipoproteins and membranes*, Elsevier.
VINODMAHATO, R., GYAWALI, P., RAUT, P. P., REGMI, P., PSD, K., SINGH, D. R. P. & GYAWALI, P. 2011. Association between glycaemic control and serum lipid profile in type 2 diabetic patients: Glycated haemoglobin as a dual biomarker. *Biomedical Research,* 22, 375-380.
VORS, C., PINEAU, G., GABERT, L., DRAI, J., LOUCHE-PÉLISSIER, C., DEFOORT, C., LAIRON, D., DÉSAGE, M., DANTHINE, S. & LAMBERT-PORCHERON, S. 2013. Modulating absorption and postprandial handling of dietary fatty acids by structuring fat in the meal: a randomized crossover clinical trial. *The American journal of clinical nutrition,* 97, 23-36.
WASSEF, G. 1999. Lipoprotein (a) in android obesity and NIDDM: a new member in 'the metabolic syndrome'. *Biomedicine & pharmacotherapy,* 53, 462-465.

WATSON, A. D., LEITINGER, N., NAVAB, M., FAULL, K. F., HÖRKKÖ, S., WITZTUM, J. L., PALINSKI, W., SCHWENKE, D., SALOMON, R. G. & SHA, W. 1997. Structural identification by mass spectrometry of oxidized phospholipids in minimally oxidized low density lipoprotein that induce monocyte/endothelial interactions and evidence for their presence in vivo. *Journal of Biological Chemistry,* 272, 13597-13607.

WHITE, A. L., RAINWATER, D. & LANFORD, R. E. 1993a. Intracellular maturation of apolipoprotein [a] and assembly of lipoprotein [a] in primary baboon hepatocytes. *Journal of lipid research,* 34, 509-517.

WHITE, A. L., RAINWATER, D. L. & LANFORD, R. E. 1993b. Intracellular maturation of apolipoprotein[a] and assembly of lipoprotein[a] in primary baboon hepatocytes. *J Lipid Res,* 34, 509-17.

WHITING, D. R., GUARIGUATA, L., WEIL, C. & SHAW, J. 2011. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. *Diabetes research and clinical practice,* 94, 311-321.

WIERINGA, G. 2000. Lipoiprotein(a): what's in a measure. *Annals of Clinical Biochemistry,* 37, 571-80.

WILD, S., ROGLIC, G., GREEN, A., SICREE, R. & KING, H. 2004. Global prevalence of diabetes estimates for the year 2000 and projections for 2030. *Diabetes care,* 27, 1047-1053.

YANO, Y., SHIMOKAWA, K., OKADA, Y. & NOMA, A. 1997. Immunolocalization of lipoprotein (a) in wounded tissues. *Journal of Histochemistry & Cytochemistry,* 45, 559-568.

YEN, C.-L. E., STONE, S. J., KOLIWAD, S., HARRIS, C. & FARESE, R. V. 2008. Thematic review series: glycerolipids. DGAT enzymes and triacylglycerol biosynthesis. *Journal of lipid research,* 49, 2283-2301.

ZIMMET, P., ALBERTI, K. & SHAW, J. 2001. Global and societal implications of the diabetes epidemic. *Nature,* 414, 782-787.

Appendix I

Appendix I
(Questionnaire)

File NO. _____ Patient Name: _____

Age: …….. . **Gender: M ( ) F ( ) Initial diagnosis:** ……. .

Height: …… **m. Weight:** …… **Kg. BMI:** …… **Kg/m^2**

Waist Cir: …… **cm. Hip Cir:** …… **cm. Waist/Hip ratio:** ……. .

Nick Cir: …….. **cm. sys BP:** …… **mmHg. Dias BP:** …… **mmHg.**

Physical activity: low ( ) moderate ( ) vigorous ( )

Smoker: Yes ( ) No ( ) Ex smoker: Yes ( ) No ( ) For ……. **Years.**

Passive smoker: Yes ( ) No ( ) For ……. **Years.**

Follow diabetic diet: Yes ( ) No ( )

Do/ Did You Have:

Hypertension ( ) CVD ( ) PVD ( ) CVD ( ) Heart disease ( )

Heart failure ( ) Arterial fibrillation ( ) Retinopathy ( ) Liver disease ( )

Acute or chronic renal failure ( ) Nephrotic syndrome ( ) Hypothyroidism ( )

Hyperthyroidism ( ) Gout ( ) Diabetic ketoacidosis ( ) Acute infection ( )

Nonketotic hyperosmolar syndrome ( ) Familial hypercholesterolemia ( )

Familial dyslipidemia ( ) Proteinuria ( ) Steroid sex hormones disorders ( )

Other diseases: …… .

Do you take:

Metformin ( ) Insulin ( ) Statins ( ) fibrates ( ) Niacin( ) Ezetimibe ( )

Neomycin ( ) aspirin ( ) L-carnitine ( ) Vit C with l-lysin ( )

Calcium antagonist ( ) A C E inhibitor ( ) Estrogen and its replacements ( )

Androgens ( ) Antiestrogens ( ) Thyroxine replacement ( ) Diuretics ( )

B- blockers ( ) Oral contraceptive ( )

Other drugs or surgery: …… .

FBS: …… mg/dl. HBA1c: …….. %. Insulin: ……. µIU/ml. TC: ……. mg/dl

HDL: ……. mg/dl. LDL: ……. mg/dl. TG: …….. mg/dl. Lp(a) …….. mg/dl .

Summary

Summary

Among the most common chronic disorders of modern time is diabetes mellitus (DM), which remains unique because of its multisystem ramifications. Type 2 diabetes mellitus is a well-known risk factor for the development of cardiovascular disease, cerebrovascular disease, and peripheral vascular disease. In addition to the established major risk factors, atherosclerosis in type 2 diabetes is related to diabetic dyslipidemia, which is characterized by high triglyceride, and LDL levels, and low HDL levels.

Lipoprotein A Lp(a) is an independent risk factor for premature CAD and other thromboembolic disorders, and many studies have reported that Lp(a) is elevated in type 2 DM. The detrimental effect of the excess of Lp(a) in atherosclerosis is probably related to the observation that Lp(a) could promote the inhibition of the the conversion of plasminogen to plasmin, thus helps thrombus formation. Lp(a) plays another role in atherosclerosis development by recruiting monocytes to the vessel wall and promoting their binding, could lead to foam cell formation. The present study designed to evaluate the association of the Lp(a) levels with type 2 diabetes mellitus in addition to its association with the degree of glycemic control, insulin, and lipid profile.

In the present study, a total of 100 type 2 Libyan diabetic patients were recruited from the Benghazi Center for Diagnosis and Treatment of Diabetes, and Alhaia Clinic in Benghazi. Thirty apparently healthy age and sex- matched individuals were selected from the high institute of medical professions, Benghazi to serve as controls. Clinical information and medical history were obtained through the review of patient medical files and patients' interviews. Venous blood samples were drawn from all the participants after at least 10 hours fasting, and analyzed for blood glucose, HbA1c, total cholesterol, triacyglycerol, and HDL-c using an autoanalyzer with enzymatic techniques. LDL cholesterol was calculated according to Friedewald equation. Serum insulin was determined by electrochemiluminescence based instrument, and Lp(a) concentration was measured by ELISA using a monoclonal antibody against Lp(a).

The data were analyzed using SPSS version 17, *P* values < 0.05 were considered to be statistically significant.

In the present study, diabetic patients had significntly higher lipoprotein (a), fasting blood glucose, insulin, total cholesterol, triacyglycerol, and LDL-c, and lower HDL-c than control patients group. Pearson's correlation analysis revealed a significant positive association between Lp(a) and total cholesterol, and LDL-c. No significant correlation were found between Lp(a) and glycemic control parameters (HbA1c, and FBS), insulin, TAG, and HDL-c.

The effect of hyperglycemia on the rate of synthesis, transcription, and translation of apo(a) is still not fully understood. The concentration of glycosylated Lp(a) is increased in the blood stream of diabetic patients. It is evident from many studies that glycosylation prolongs the half-life of lipoproteins and this may be applicable for Lp(a), which may result in higher levels of Lp(a) in diabetic patients. Plasma Lp(a) concentrations are primarily regulated at the gene level , and an inverse correlation has been found between plasma Lp(a) concentration and the size of Apo(a).

The association of Lp(a) levels with DM has been a matter of some controversies. The major reasons for the inconsistent results of the prospective studies have been attributed to the variation in study design, collection and storage of samples, methods used for statistical analysis and population differences that reflect the known ethnic variability in the Apo(a) size isoforms and distribution of plasma Lp(a) levels.

الخلاصة

داء السكري هو من بين الاضطرابات الصحية المزمنة الأكثر شيوعا فى الزمن الحديث، و التي تعتبر فريدة من نوعها بسبب تداعياتها المتعددة على مختلف أجهزة الجسم. بالإضافة إلى عوامل الخطر الرئيسية المعروفة داء السكري نوع 2 هو أحد عوامل الخطر المؤكدة لتطور أمراض القلب والأوعية الدموية والأمراض الدماغية الوعائية، وأمراض الأوعية الدموية الطرفية. ويرتبط تصلب الشرايين في مرض السكري النوع 2 بإضطرابات الدهون الناتجة عن مرض السكري، والتي تظهر فيها الدهون الثلاثية و مستويات LDL مرتفعة. بينما مستويات HDL منخفضة.

يعتبر البروتين الدهني (أ) عامل خطر مستقل و مبكر لمرض الشرايين التاجية وللإضطرابات الانصماممية الخثارية الأخرى، كما أن العديد من الدراسات بينت أن إرتفاع البروتن الدهنى (أ) يصحب ظهور داء السكري نوع 2. ويعزى تأثير ارتفاع البروتين الدهني (أ) الضار المسبب لتصلب الشرايين إلى الملاحظة أن البروتين الدهني (أ) يمكن أن يعزز تثبيط تحويل البلازمينوجين إلى بلازمين، بالتالي يساعد تشكيل الخثرات. كما أن له دور آخر في تطور تصلب الشرايين عن طريق استدعاء خلايا الدم البيضاء المعروفة بوحيدة الخلية إلى جدار الوعاء الدموي و تعزيز إلتصاقها به، والتي يمكن أن تؤدي إلى تشكيل الخلايا الرغوية.

هذه الدراسة صممت لتقييم ارتباط مستويات البروتين الدهني (أ) مع داء السكري النوع 2 بالإضافة إلى ارتباطه مع درجة التحكم في السكر، والأنسولين، ومستويات الدهون في الدم.

في هذه الدراسة تم الحصول على ما مجموعه 100 عينة من المرضى الليبيين المصابين بداء السكري النوع 2 من مركز بنغازي لتشخيص وعلاج مرض السكري، بالإضافة إلى عيادة الحياة في بنغازي. كما تم الحصول على 30 عينة من أشخاص اصحاء ومطابقين للمرضى من حيث العمر و الجنس من المعهد العالي للمهن الطبية، بنغازي لتكون بمثابة الضوابط.

تم الحصول على المعلومات السريرية والتاريخ الطبي من خلال استعراض الملفات الطبية للمرضى والمقابلات مع المرضى و الحالات الضابطة. تم سحب عينات من الدم الوريدي من جميع المشاركين بعد 10 ساعات على الأقل من الصيام، ومن ثم تحليل مستوى السكر في الدم، نسبة HbA1c، والكولسترول الكلي، والدهون الثلاثية، والدهون ذات الكثافة العالية (HDL-c) باستخدام المحلل الاتوماتيكي بتقنيات أنزيمية. أما بالنسبة للدهون ذات الكثافة المنخفضة (LDL-c) فقد تم احتسابها وفقا لمعادلة Friedewald. تم تحديد الأنسولين في الدم بواسطة electrochemiluminescence، أما البروتين الدهنى (أ) فقد تم قياس تركيزه عن طريق ELISA. وقد تم تحليل البيانات باستخدام SPSS النسخة 17، وقيم P أصغر من 0.05 تعتبر ذات دلالة إحصائية.

في هذه الدراسة كان معدل البروتين الدهني (أ) أعلى إحصائيا عند مرضى السكري، كما هو الحال كذلك لمعدل الجلوكوز في الدم، والانسولين والكوليسترول الكلي، والدهون الثلاثية، والدهون منخفضة الكثافة (LDL-c)، أما

الدهون مرتفعة الكثافة (HDL-c) فقد سجلت انخفاض مقارنة بمجموعة الضوابط. أما تحليل ارتباط بيرسون فقد بين وجود علاقة إيجابية ذات دلالة إحصائية بين البروتين الدهني (أ) و الكوليسترول الكلي، والدهون منخفضة الكثافة. كما أنه لا توجد علاقة ذات دلالة إحصائية بين البروتين الدهني (أ) و قياسات التحكم في مستوى السكر في الدم (HbA1c، وFBS)، والانسولين، و TAG، و HDL-c.

ويعتبر تأثير ارتفاع السكر في الدم على معدل انتاج البروتين الدهني (أ) لا يزال موضع بحث وغير مفهوم بشكل كامل, ومن المعلوم أن البروتين الدهني (أ) يزداد تركيزه في مجرى الدم لمرضى السكري، ويتضح من العديد من الدراسات أن إرتباط الجلوكوز بالبروتينات يطيل فترة نصف العمر للبروتين، وهذا ينطبق على البروتين الدهني (أ)، مما قد يؤدي إلى مستويات عاليه منه في مرضى السكري. يتم تنظيم تركيز البروتين الدهني (أ) في الدم في المقام الأول على مستوى الجينات، بالإضافة إلى أنه قد ثبت وجود علاقة عكسية بين مستوى البروتين الدهني (أ) في البلازما و حجم هذا البروتين.

ويعتبر الإرتباط بين البروتين الدهني (أ) وداء السكري النوع 2 هو مسألة خلافية، وقد نسبت الأسباب الرئيسية لهذا الخلاف الى النتائج المتحصل عليها من دراسات مصممة بشكل غير متناسق، وكذلك طرق تجميع وتخزين العينات والطرق المستخدمة في تحليل العينات والنتائج، كما أن الإختلاف العرقي لسكان المنطقة المستخدمة في البحث قد تعكس التباين الوارد بين هذه الأبحاث.

كلية الطب البشري

عنوان الرسالة:

البروتين الدهني (أ) باعتباره عامل خطر لتصلب الشرايين في المرضى المصابين بداء السكري النوع 2 في ليبيا

مقدم من:

عمر بالحسن سالم عبدالله الأطيوش

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أ.د فرج علي الشاعري

المشرف المساعد:

أ.د محمد حمزة سليمان

قُدمت هذه الرسالة استكمالاً لمتطلبات درجة الماجستير في الكيمياء الحيوية

بكلية الطب البشري/ جامعة بنغازي.

2015 - 2014