"A CORRELATION STUDY OF SERUM LIPOPROTEIN (a) LEVEL AND THE DEVELOPMENT OF VASCULAR COMPLICATIONS IN TYPE 2 DIABETES MELLITUS PATIENTS"

By

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Dissertation submitted to the
KLE University, Belgaum, Karnataka

In Partial Fulfillment
of the requirements for the degree of

M. D. MEDICINE

Under the Guidance of

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MAY - 2009

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I hereby declare that this dissertation entitled **"A CORRELATION STUDY OF SERUM LIPOPROTEIN (a) LEVEL AND THE DEVELOPMENT OF VASCULAR COMPLICATIONS IN TYPE 2 DIABETES MELLITUS PATIENTS"** is a bonafide and genuine research work carried out by me under the guidance of **Dr. V. A. Kothiwale** **MD, Ph.D** Professor and Head, Department of Medicine, Jawaharlal Nehru Medical College, Nehru Nagar, Belgaum-590010.

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LIST OF ABBREVIATIONS USED

ABC-1	-	ATP Binding Cassette Transporters
ADA	-	American Diabetic Association
AGE	-	Advanced Glycation End Products
Apo (a)	-	Apolipoprotein (a)
Apo (B)	-	Apolipoprotein B
ATP	-	Adenosine Triphosphate
CE	-	Cholesteryl Ester
CETP	-	Cholesterol Ester Transfer Protein
CAD	-	Coronary Artery Disease
CHD	-	Coronary Heart Disease
CVD	-	Cardio Vascular disease
DM	-	Diabetes Mellitus
EL	-	Endothelial Endothelial Lipase
ESRD	-	End Stage Renal Disease
FC	-	Free Cholesterol
Glu PLG	-	N Terminal Glutamic Acid Plasminogen
HL	-	Hepatic Lipase
HDL	-	High Density Lipoprotein
HSL	-	Hormone Sensitive Lipase
HSPG	-	Heparin Sulfate Proteoglycan
IDL	-	Intermediate Density Lipoprotein
IHD	-	Ischaemic Heart Disease
IRMA	-	Intraretinal Microvascular Abnormalities

K-IV	-	Kringle IV
K-V	-	Kringle V
L-NMMA	-	Low concentration of NG-monomethyl-L arginine monoacetate
LCAT	-	Lecithin Cholesterol Acyl Transferase
LDL-R	-	Low Density Lipoprotein Receptor
LDL-c	-	Low Density Lipoprotein Cholesterol
Lp (a)	-	Lipoprotein (a)
LPL	-	Lipoprotein Lipase
LRP	-	Lipoprotein Receptor Protein
Lys-PLG-N	-	N Terminal Lysine Plasminogen
MI	-	Myocardial Infarction
NCEP	-	National Cholesterol Education Programme
NFκB	-	Nuclear Factor Kappa B
NIDDM	-	Non Insulin Dependent Diabetes Mellitus
NVD	-	Neovascularisation Disc
NVE	-	Neovascularisation Elsewhere
PAD	-	Peripheral Arterial Disease
PAI-1	-	Plasminogen Activator Inhibitor 1
PDR	-	Proliferative Diabetic Retinopathy
PL	-	Phospholipid
PL TP	-	Phospholipid Transfer Protein
RAGE	-	Receptor for Advanced Glycation End Product
SR – B1	-	Scavenger Receptor B1
TGF β	-	Transforming Growth Factor β

tPA	-	tissue Plasminogen Activator
UK	-	United Kingdom
UKPDS	-	United Kingdom Prospective Diabetes Study
US	-	United States
VCAM 1	-	Vascular Cell Adhesion Molecule 1
VNTR	-	Variable Number Tandem Repeats

ABSTRACT

Background and Objectives

Diabetes is the most common endocrine disorder and a burdensome chronic disease in the world today. The objective of the study is to assess the vascular complications of type 2 DM with respect to their serum levels of Lp (a).

Methodology

The present one year correlation study was conducted in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum on 50 patients diagnosed to have type 2 DM more than 10 years, proved by laboratory reports during the period of January 2007 to December 2007. A detailed history, physical examination, limited vascular and neurological examinations were done. Serum lipoprotein (a) levels were measured by randox immunoturbido-metric immunoassay. Fasting lipid profile and blood sugar levels along with other relevant investigations were also done.

Results

The results of the study showed that, Lp (a) values progressively increased as age increased. Diabetic patients with microvascular complications like retinopathy, nephropathy and neuropathy had increased levels of Lp (a). The present study indicated that better the glycemic control, lesser the mean Lp (a) levels. As the blood sugars and other lipid levels increased, Lp (a) levels were also increased except HDL wherein an inverse proportion was observed. Lp (a) levels may be used as a screening or predictive tool for development of coronary artery disease.

Conclusion and interpretation

Lp (a) may be an independent risk factor for the development of different microvascular and macrovascular complications in type 2 diabetes mellitus.

Keywords

Lipoprotein (a); Diabetes mellitus; Retinopathy; Nephropathy; Neuropathy;

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INTRODUCTION

Diabetes is the most common endocrine disorder and a burdensome chronic disease in the world today.

Dyslipidemias frequently associated with type 2 diabetes mellitus (DM) are hypertriglyceridemia, hypercholesterolemia, postprandial lipemia, low high density lipoprotein (HDL), preponderance of small and dense low density lipoprotein (LDL), and elevated levels of Lipoprotein (a) [Lp (a)].[1]

Among dyslipidemias associated with type 2 DM elevated levels of serum Lp (a) is of much importance, since it is an important risk factor for atheromatous complications in diabetics than compared to non diabetics.

Recently, much interest has been focused on Lp (a) which is a plasma complex composed of apolipoprotein (a) [Apo (a)] covalently linked to Apo B-100 by disulfide bridges. Because of the structural similarity of Apo (a) to plasminogen, Lp (a) has been suggested to have antifibrinolytic properties.

Diabetic patients are reported to have higher Lp (a) levels than non diabetic persons.[2] The data on relationship between Lp(a) and diabetes is scarce and the data on Lp (a) in Asian Indian diabetics is still meager.

There are studies which have conclusions that Lp (a) is an independent risk factor for coronary artery disease[2] and is a reliable predictor of coronary artery disease severity in type 2 DM patients.[3]

Lipoprotein (a) is also a risk factor for the progression of diabetic nephropathy with overt proteinuria,[4] arterial stiffness in elderly patient and peripheral arterial disease in type 2 DM patients.[5]

High serum Lp (a) plays a major role in the occlusion of retinal capillaries leading to proliferative diabetic retinopathy.

Therefore the present study has been undertaken to assess the serum concentration of Lp (a) and the onset and progression of different microvascular and macrovascular complications which develop in type 2 DM patients. This will enable the clinicians to roughly predict the time of development of complications in type 2 DM based on their serum Lp (a) levels.

OBJECTIVES

The objective of the study is to assess the vascular complications of type 2 DM with respect to their serum levels of Lp (a).

Hypothesis

Different levels of serum Lp (a) is associated with microvascular and macrovascular complications of type 2 DM.

REVIEW OF LITERATURE

LIPOPROTEIN (a) [Lp (a)]

Lipoprotein (a) was first described in 1963 by Kare Berg. Lp (a) was considered to be an autosomal dominant trait but subsequent work using quantitative immunochemical methods have shown that Lp (a) represents a quantitative rather than a qualitative genetic marker whose concentration can vary enormously between different individuals.

Biology and genetics

Lipoprotein (a) is a cholesteryl-ester-rich lipoprotein of unknown function formed by the covalent disulfide linkage of Apo (a) to Apolipoprotein B [(Apo B)] of low density lipoprotein cholesterol (LDL-c). Apo (a) is a glycoprotein with approximately 29% carbohydrates and resembles plasminogen, the plasma zymogen for plasmin.[6] The Apo (a) gene contains between 12 and 51 copies of a deoxyribose nucleic acid (DNA) sequence encoding a tandemly repeated cysteine-rich motif called kringle IV (K-IV), which is found in one copy in the plasminogen gene. Apo (a) exists in multiple genetically determined isoforms with molecular weights ranging from approximately 350 to 1000 kDa. The number of K-IV repeats is inversely correlated with the plasma Lp (a) concentration.

Structure difference between Lp (a) and Apo (a)

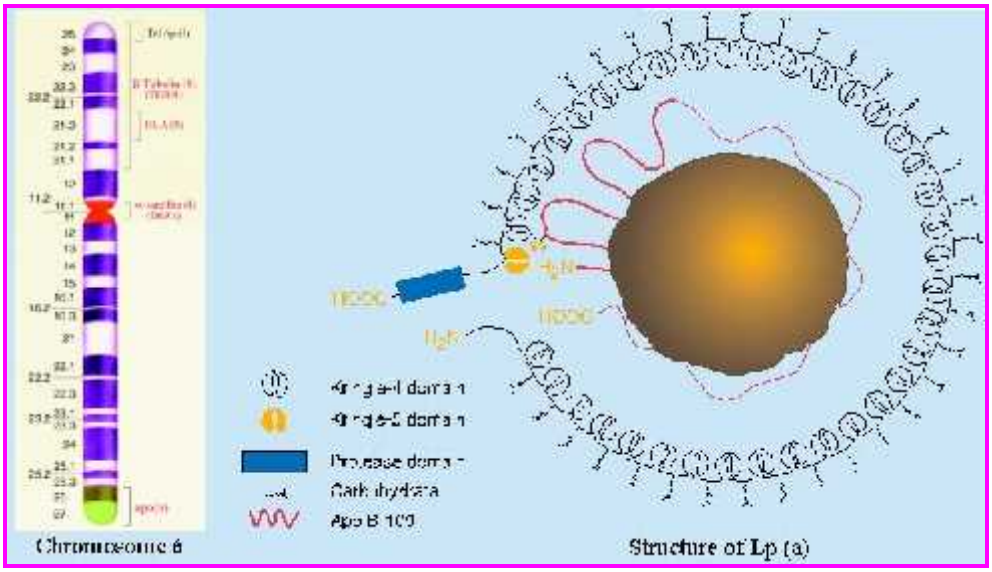


Figure No. 1: Structure of lipoprotein (a)

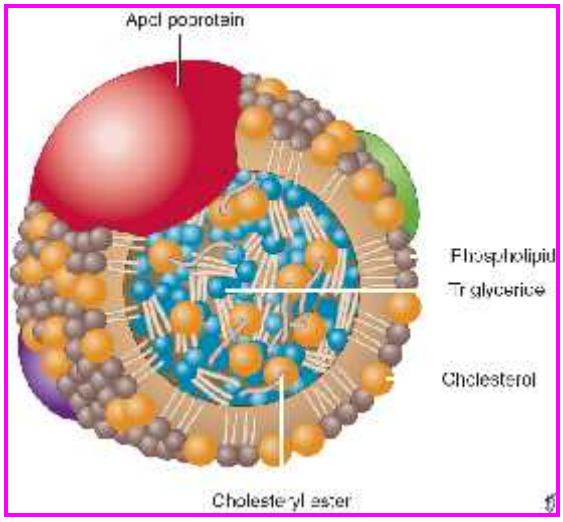


Figure No. 2: Structure of low density lipoprotein

The Apo (a) and complementary plasminogen deoxyribose nucleic acid (cDNAs) have been cloned and the two genes are closely linked on chromosome 6 (q26-27).[7] Analysis of the Apo (a) cDNA revealed a characteristic structure consisting of numerous K-IV repeats, one Kringle V (K-V), and a protease domain. These are homologous to corresponding structures in plasminogen mimicking its functional properties as well. This could partially explain the thrombogenic properties of Lp (a).[8]

Genetic size polymorphism of Apo (a) has been demonstrated at the protein, messenger ribose nucleic acid (mRNA) and DNA levels. This polymorphism of Apo (a) was originally identified by Utermann and his co-workers by sodium dodecyl sulfate agarose polyacrylamide gel electrophoresis. The isoforms were designated as F, B, or S based on the pattern of mobility that is either faster equal to or slower than Apo B-100. Sophisticated separation techniques such as sodium dodecyl sulfate agarose gel electrophoresis have allowed identification of more than 34 Apo (a) size isoforms. There exists a perfect correlation between the size of Apo (a) DNA fragments, size of protein isoforms and Apo (a) mRNA size. Information on both the size and the degree of expression of an allele is obtained by performing Apo (a) DNA and protein typing. The Apo (a) gene locus is thus best described as a transcribed and translated variable number tandem repeats (VNTR) locus that determines the extensive variation in size and concentration of the Apo (a) protein.

Structure – function relationship of lipoprotein (a) and plasminogen

Plasminogen is proteolytically inactive until it has been cleaved by tissue plasminogen activator (tPA). However, despite containing a homologous protease domain, Apo (a) is unlikely to possess proteolytic activity as a single nucleotide variation in the Apo (a) gene sequence resulting in a serine-arginine substitution in the amino acid sequence of Apo (a) at the precise cleavage site for tPA precludes its activation to a protease. In addition, Apo (a) from a number of individuals has failed to exhibit any plasmin-like activity, despite treatment with tPA, urokinase and streptokinase.

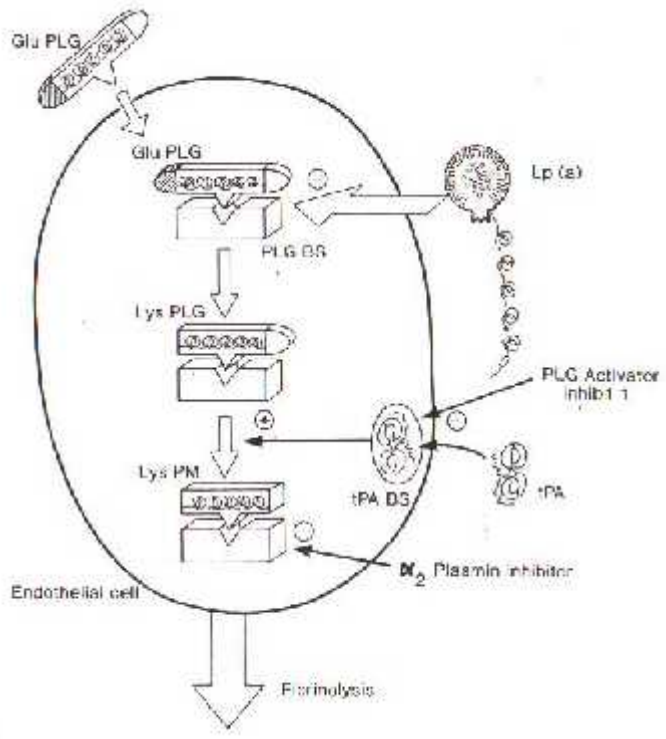


Figure No. 3: Schematic representation of the fibrinolytic system on the endothelial cell surface - negative sign denotes inhibitory effect and positive sign denotes facilitatory effect

A number of studies have addressed this possibility and conclude that Lp (a) competes with plasminogen for its binding site in both a dose-dependent and K-IV dependent manner but has no effect on fluid phase plasmin.[9] The physiological relevance of these observations obviously requires further evaluation, but the available evidence suggests that high levels of Lp (a) may alter the delicate balance between profibrinolytic and antifibrinolytic activities in maintaining homeostasis by competing via molecular mimicry with plasminogen for binding sites at the endothelial cell surface. Supporting this hypothesis are the epidemiological data showing that the 20% of the population with levels of Lp (a) greater than 30 mg/dL have a two fold increase in relative risk of developing coronary atherosclerosis. If both Lp (a) and LDL cholesterol levels arc elevated, the risk rises to four to five fold.[9]

Using in vitro data from studies on cell cultures, it is estimated that plasma concentrations of Lp (a) of 30 mg/dL would reduce plasminogen binding by 20%, thus encouraging a relatively procoagulant state.[9] Further circumstantial evidence implicating Lp (a) in atherogenesis is the identification of a striking accumulation of Lp (a) immunoreactive material associated with the endothelium and intima of atherosclerotic coronary arteries from autopsy specimens and from the arterial wall of coronary artery bypass patients.[9]

This immunoreactive material has been proven to be Apo (a) related. Medium-sized arteries with minimal atherosclerotic disease revealed no detectable staining for Lp (a) suggesting that at least in some individuals, Lp (a) accumulates in the vessel wall of atherosclerotic arteries. This may be due to increased binding of Lp (a) to macrophages or arterial wall structures, or it may

be secondary to inhibition of fibrinolysis. Whatever the explanation, these tantalizing associations inevitably lead to speculation that Lp (a) provides a bridge between lipid metabolism and thromboregulation at the endothelial surface of the vessel wall, thus providing a potential explanation for the increased thrombotic risks associated with elevated levels of Lp (a).

Table No. 1: Composition of lipoproteins in body[10]

	Origin	**Density (gm/mL)**	**Size (nm)**	**% Protein**	**Cholesterol in plasma**	**Triglyceride in fasting plasma**	**Major apo**	**Other apo**
Chylomicrons	Intestine	< 0.95	100-1000	1 -2	0.0	0	B48	AL C's
VLDL	Liver	< 1.006	40-50	10	0.1-0.4	0.2-1.2	B100	AL C's
IDL	VLDL	1.006-1.009	25-30	18	0.1-0.3	0.1-0.3	B100, E	
LDL	IDL	1.019-1.063	20-25	25	1.5-3.5	0.2-0.4	B100	
HDL	Tissues	1.063-1.210	6-10	40-55	0.9-1.6	0.1-0.2	Al	All, AIV
LP (a)	**Liver**	**1.051-1.082**	**25**	**30-55**	**-**	**-**	**B100, (a)**	

Metabolism of lipoprotein (a)

The largely varying plasma concentration of Lp (a) is randomly distributed in the population and correlates inversely with the molecular mass of

Apo (a). This protein is shown to have an unusual secretory pathway, mostly derived from studies of the intracellular metabolism of Apo (a) in transfected human hepatoma cells and in primary baboon hepatocytes.[11] It is known that Apo (a) is synthesized in the liver. In vivo turnover studies have revealed that variations in the plasma levels of Lp (a) are due to its synthesis rather than the degradation. An immature precursor form of Apo (a) is retained in the endoplasmic reticulum for a prolonged time due to complex folding and processing. Since the retention time correlates positively with the Apo (a) isoform size, this intracellular mechanism could explain the inverse correlation between the isoform size and the plasma concentrations. Another unusual feature of the biogenesis of Lp (a) is that the mature Lp (a) complex is formed only following separate secretion of Apo (a) and LDL-like particles. Upon secretion from hepatocytes, Apo (a) is assembled with plasma low density lipoprotein cholesterol (LDL-c) to form Lp (a).[12]

This process requires docking and formation of a single disulfide bond between Apo B-100 in LDL-c and K-IV in Apo (a). The metabolism of Lp (a) is independent of other lipoproteins. The major source of circulating plasma Lp (a) is the human liver. Serum Lp (a) concentration is determined by the rate of Lp (a) production and correlates directly with hepatic mRNA abundance. Studies in primary cultures of baboon hepatocytes showed that the majority of Apo (a) is secreted by liver cells into the medium in its free form. A study showed that Apo (a) is the human liver is not associated with Apo B-100 and occurs extracellularly after secretion. The assembly of Apo (a) and LDL-c, which is determinant for plasma Lp (a) levels, takes place extracellularly and requires specific structural motifs in Apo (a) and Apo B.

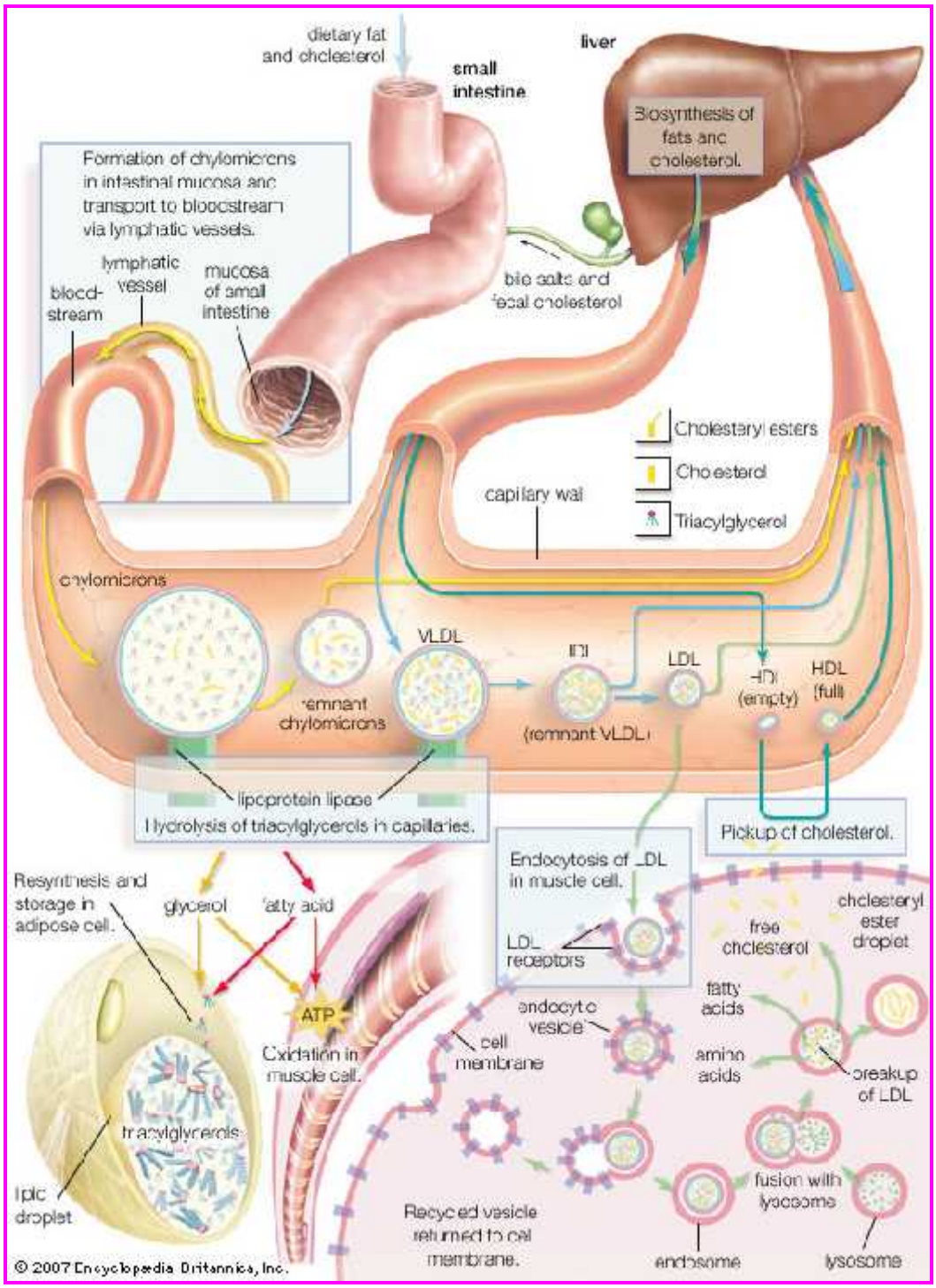


Figure No. 4: Synthesis of Lp (a) is similar to synthesis of lipoprotein complexes in the small intestine, liver, and blood plasma and their delivery to peripheral tissues of the body[10]

Lipoprotein (a) or Apo (a) receptors on macrophages, receptor-related protein, and the asialoglycoprotein receptor have been implicated. However, catabolism of Lp (a) was not significantly reduced in patients with defective LDL – receptor (LDL-R) function, suggesting that the LDL-R is not directly responsible for Lp (a) clearance in vivo. Further, recent studies suggest that mouse embryo fibroblasts do not take up Lp (a) via the LDL-R.[13] Lp (a) turnover studies have shown that approximately 70% of the Apo (a) component of Lp (a) may be released in the circulation, and the rest is degraded via the LDL-R.[14]

A study has demonstrated the presence of two distinct structural domains in Apo (a) linked by a flexible and accessible region located between K-IV-IV and K-IV-V.[15] Authors isolated the Lp (a) particle following removal of the N-terminal domain by proteolytic cleavage; the residual particle (containing the C-terminal domain sparing the region from K-IV-V to the protease domain), is linked to Apo B-100 by disulfide linkage, termed 'mini Lp (a)'. The observation that mini-Lp (a) exhibits the same binding affinity to fibrin as the corresponding Lp (a) suggests that the kringles responsible for fibrin binding are restricted to K-IV-V to K-IV-X. This observation is supported by failure of the N-terminal domain to bind to fibrin. N-terminal fragments of Apo (a) have been detected in the urine of normal subjects, thereby indicating that, part of the catabolism of Lp (a), which is largely indeterminate, could occur via the renal route.[15] This possibility is supported by the observation that the excretion of Apo (a) fragments was lesser in patients with reduced renal function.[16] Collagenase digestion of Lp (a) released Apo (a) fragments of similar size to those found in urine, producing a particle that could then bind to LDL-R.[17]

Lipoprotein (a) estimation

Laboratory estimation is performed by radioimmunoassay, immuno electrophoresis and using monoclonal antibodies. Antibodies are raised against either Lp (a) or Apo (a) in the intact Lp (a) molecule since dissociation of Apo (a) from Apo B-100 decreases its immuno reactivity. If antibodies are raised against K-IV repeats, it would lead to heterogeneity due to variation in their numbers. A recent report suggests that patients suffering from coronary artery disease (CAD) excrete significantly higher amounts of Apo (a) into the urine than controls and that urinary Apo (a) is a valuable predictor of CAD. Using urinary Apo (a) as a marker for CAD has the advantage of easier sampling compared to plasma samples.[18]

Apolipoprotein (a) size polymorphism, lipoprotein (a) concentration and ethnic variation

Apolipoprotein (a) protein, mRNA and DNA size polymorphism studies demonstrate that the number of K-IV repeats in the gene and the resulting size of the protein are inversely correlated with Lp (a) levels in the plasma in all populations studied so far. Mean Lp (a) concentrations and Apo (a) isoform frequencies vary significantly between populations. The plasma concentration of Lp (a) has a skewed distribution that varies over a 1000 fold range in white populations, with most individuals having low plasma Lp (a). In whites, studies in sibpairs indicate that over 90% of the individual variation of the Apo (a) gene may be responsible for it, however, in other ethnic groups the contributions of this locus may be smaller.[19] A pentanucleotide sequence repeat polymorphism

(TTTTA) at position-1373 before the translation initiation codon of the Apo (a) gene may be one of the factors associated with variable plasma Lp (a) concentration. This polymorphism could account for about 10–14% of the inter-individual variations of Lp (a) levels in Caucasians.[20,21]

Other factors affecting plasma levels of lipoprotein (a)

Lipoprotein (a) levels, unlike LDL-c and Apo B levels, do not vary with the age of the subject. Lp (a) is fully expressed in the first year of life. Hence, its tracking is more useful than other lipids. Various endogenous and exogenous factors affect Lp (a) levels in humans. These include hormones (growth hormone, estrogens), hypothyroidism, and alcohol consumption. Several renal diseases alter Lp (a) levels including renal failure and nephritic syndrome. Successful renal transplantation leads rapidly to correction of Lp (a) concentrations, especially in patients treated with chronic ambulatory peritoneal dialysis with higher Lp (a) levels.

Factors influencing serum lipoprotein (a) concentrations

Physiological factors

- Age, ethnic groups, menopause, high saturated fat diet.

Chemical compounds and drugs

- Estrogen, progesterone, danazol, growth hormone, neomycin, niacin, alcohol, cyclosporine.

Diseases

- Myocardial infarction (MI), renal failure, nephrotic syndrome, familial hypercholesterolemia, rheumatoid arthritis.

Lp (a) levels are not readily amenable to manipulation by dietary restriction. However, in one of the studies, substitution of a baseline diet of polyunsaturated fat with medium-chain saturated fat led to reduction in Lp (a) levels by 30%.[22] Other recent studies indicate that Lp (a) level is lowered by saturated fat (e.g. palmitic acid) diet.[23] Moderate drinking of alcohol lowers plasma Lp (a) levels. If the alcohol was withheld from moderate drinkers, this led to increased Lp (a) levels by 64%. This may be an additional mechanism by which moderate alcohol drinking may be beneficial for atherosclerosis.

Pathogenic effects of lipoprotein (a) in atherogenesis

It is well documented that Lp (a) is present in the arterial wall at the sites of atherosclerotic lesions and that it accumulates at these sites to an extent proportional to plasma Lp (a) levels.[2] Based on this localization in the vessel wall, it has been hypothesized that the role of Lp (a) in the atherosclerotic process may depend on events that occur within the intima. In this milieu, the pathogenic role of Lp (a) may be function of its proatherogenic nature, resulting from its similarity to LDL, or its prothrombotic nature, resulting from its similarity to plasminogen. The evidence accumulated to date suggests that Lp (a) is involved in both of these processes, thereby providing a functional link between atherosclerosis and thrombosis.

Within the intima, Lp (a) can interact with various tissue matrix components, including glycosaminoglycans, fibrinogen, fibrin, and fibronectin. In addition to providing a means of retaining Lp (a) within the vessel wall, the interaction of Lp (a) with extracellular matrix components would allow Lp (a) to

become concentrated at the site of vessel injury, where it could provide an additional source of cholesterol. Once entrapped in the vessel wall, Lp (a) may then undergo oxidative modification and become a ligand for the scavenger receptor pathway in a manner similar to that of oxidized LDL. In this way, Lp (a) may effectively contribute to the formation of foam cells, thereby underscoring its atherogenic nature. It has also recently been demonstrated that native, Lp (a) can be internalized and degraded via a different and novel receptor present on cholesterol ester loaded macrophages.[24] The nature of this receptor is currently unknown.

The endothelium-dependent vasoconstrictive response to low concentration of N^G monomethyl – L – arginine mono acetate (L-NMMA) was enhanced in subjects with relatively high Lp (a) plasma levels, suggesting an increased basal production and release of nitric oxide and endothelial dysfunction in diabetes.[25] Hence oxidized Lp (a) causing endothelial dysfunction and diabetes perse causing endothelial dysfunction produces an additive effect in initiation of atherogenic disease.

In induction of atherogenesis, recent evidence indicates that Lp (a) involves adhesion molecules. An endothelial cell-activating effect of Lp (a) is potent surface expression of vascular cell adhesion molecule-1 (VCAM-1) and E-selectin. This may be an important event in the initiation of atherogenic disease.[26]

An additive effect has been demonstrated between Lp (a) and LDL in the development of angiographically detectable coronary artery disease. However, elevated Lp (a) levels were no longer atherogenic if LDL levels were

substantially decreased, suggesting that Lp (a) may not be a primary causative agent in atherogenesis.

Interestingly, human studies have lent little support for a role of Lp (a) in thrombogenic disorders. Several reports have failed to demonstrate a relation between plasma Lp (a) levels and various fibrinolytic parameters, such as euglobulin clot lysis time and levels of fibrin degradation products or α2-antiplasmin.[27] However, this does not exclude a potential role for Lp (a) in localized regions of vascular injury where the effective Lp (a) concentration is many-fold higher than in plasma, owing to the selective retention of Lp (a) in the intima.

In this study, binding of Lp (a) to cultured endothelial cells resulted in the upregulation of plasminogen activator inhibitor- 1 secretion, with a concomitant increase in inhibitor activity. No increase in tissue-type plasminogen activator transcription or activity was observed, suggesting that increased plasminogen activator inhibitor-l levels induced by Lp (a) may produce impaired activation of plasminogen in vivo.[28]

Another study has demonstrated that Lp (a) interferes with the production and/or secretion of tissue type plasminogen activator from endothelial cells, which could lead to impaired plasminogen activation and inhibition of fibrinolysis on endothelial cells in vivo.[29]

Furthermore, it has been shown that accumulation of Lp (a) in the vessel wall at the sites of developing atheromas can increase adhesion of circulating monocytes to the endothelial cell surface;[30] and that Lp (a) and Apo (a), but not

plasminogen, can stimulate monocyte chemotactic activity in endothelial cells.[31] Taken together, these observations suggest several potential mechanisms by which Lp (a) can mediate its pathogenic effects via interactions with the vascular endothelium. This finding further suggests that thromboembolic complications may be mediated by Lp (a) in vivo.

Association of Lp (a) and CAD was first observed in 1974. The accumulated data have established it as an important inherited risk factor for the macrovascular diseases including CAD, cerebrovascular and peripheral vascular diseases.

Several case-control studies have demonstrated an association of elevated Lp (a) plasma concentrations with premature coronary atherosclerosis and myocardial infarction.[32] Lp (a) is considered to be ten times more atherogenic than LDL-c. Relative risk of CAD is increased three-fold if the levels of Lp (a) are more than 30 mg/dL.[9] Serum Lp (a) levels have been shown to correlate well with the presence, extent, severity and score of atherosclerotic lesions on coronary angiography. The Scandinavian Simvastatin Survival Study provides independent confirmation that a high Lp (a) lipoprotein level is a significant CAD risk factor.[33] However in Quebec Cardiovascular Study, Lp (a) was not an independent risk factor for CAD but appeared to increase the risk associated with other lipid risk factors.[34] The pathogenic association of Lp (a) and CAD has been further emphasized in a symposium on this topic held in Oslo in May 1997.[35]

The risk attributable to Lp (a) in atherogenesis may be dependent upon or enhanced by the presence of facilitating factors such as dyslipidemia and diabetic

dyslipidemia confers an additive risk in the development of CVD. Patients with diabetic dyslipidemia are at increased risk of developing macrovascular and microvascular complications. Hence Lp (a);

- Contributes to uptake of LDL-c and formation of foam cells.
- Inhibits plasminogen activation and fibrinolysis leading to procoagulant tendency.
- Release of cytokines.
- Release of growth factors, smooth muscle cell proliferation.
- Increased expression of adhesion molecules.
- Endothelial dysfunction.
- Interacts with other risk factors for example homocysteine.

Diabetic dyslipidemia

In dyslipidemia, oxidized Lp (a) is implicated in the causation of endothelial dysfunction and endothelial dysfunction perse caused by diabetes has an additive effect on it.

The characteristic pattern of lipoproteins in type 2 diabetes includes an increase in triglycerides and a decrease in HDL cholesterol. Concentrations of LDL cholesterol do not differ significantly from concentrations found in nondiabetic individuals but are predominated by the small dense form of LDL. The small dense LDL particles are more intrinsically atherogenic than the normal larger and more buoyant LDL particles.

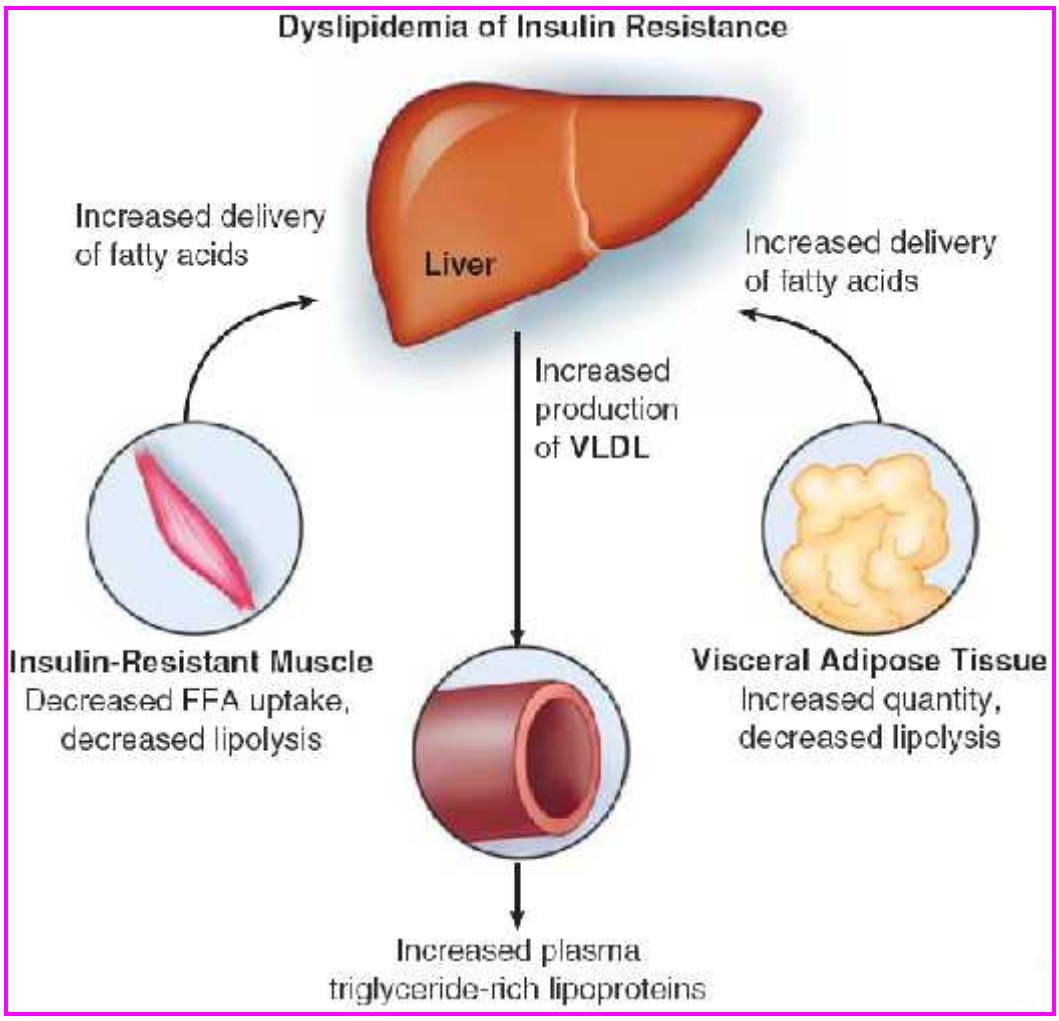


Figure No 5: Diabetic dyslipidemia

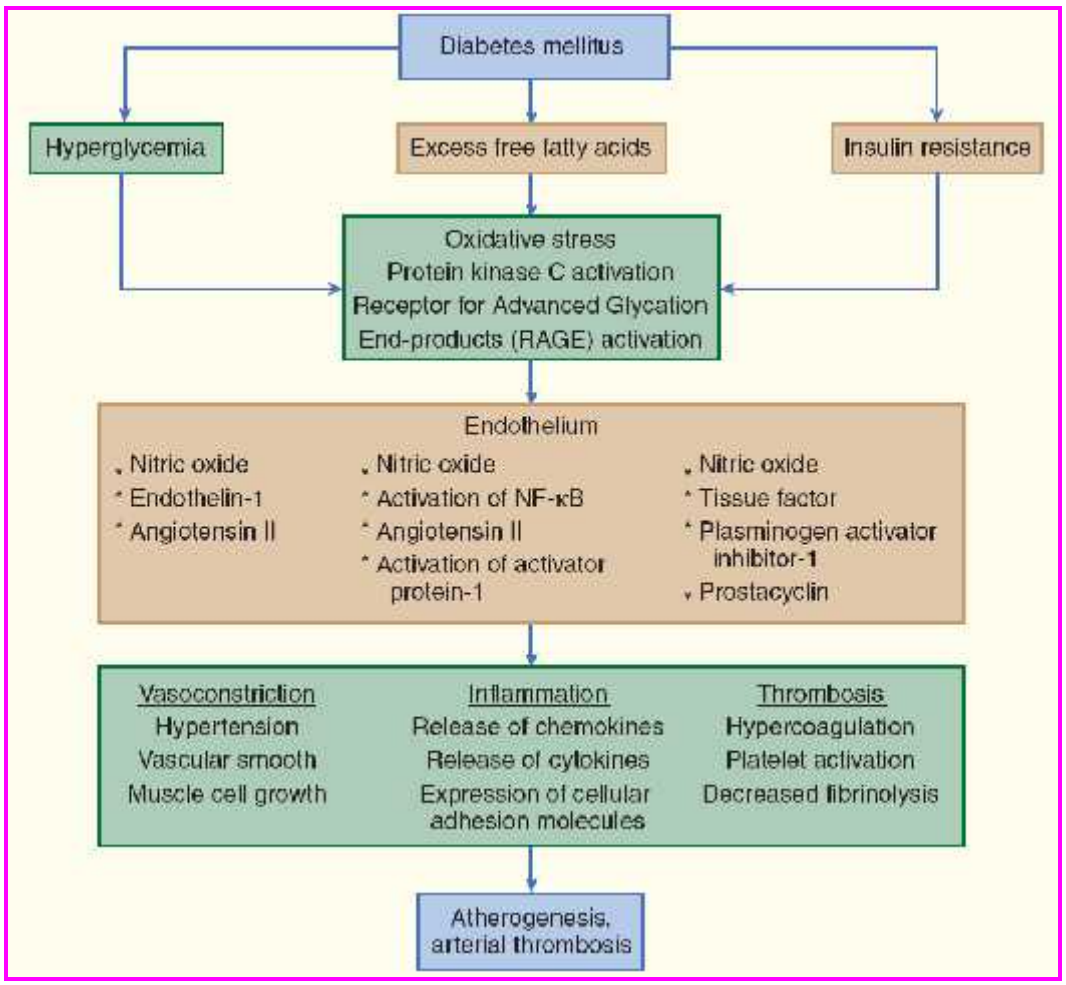


Figure No. 6: Pathogenesis of atherogenesis in diabetes mellitus patients

Furthermore, because of their smaller mass, a greater number of LDL particles is contained within the plasma of patients with small dense LDL, further increasing atherogenic risk. This triad of lipid abnormalities has been termed "diabetic dyslipidemia". The presence of diabetic dyslipidemia confers a CVD risk estimated to be equivalent to an LDL cholesterol concentration of 150 to 220 mg/dl.[36]

Glycosylation, oxidation, and triglyceride enrichment of lipoproteins contribute to the observed increase in atherogenicity. Glycosylation of LDL increases its half life, causes it to be the more atherogenic small dense variety, and makes it more likely to be oxidized and taken up by macrophages to form foam cells.[37] Glycosylation of HDL cholesterol shortens its half-life and causes the less protective HDL_3 to predominate over the more protective HDL_2 form of the lipoprotein.[38] Triglyceride enrichment leads to increased production of the small dense form of LDL cholesterol and to depletion of HDL cholesterol. The ability of HDL to transport cholesterol from peripheral tissues back to the liver may be decreased when HDL is triglyceride enriched.[39] Improvement in blood glucose control as a consequence of lifestyle change or treatment with insulin and an oral antidiabetic agent leads to decreased triglyceride levels, increased HDL levels, decreased glycosylation of lipoproteins, and decreased triglyceride enrichment of lipoproteins.

Defects in insulin action and hyperglycemia could lead to changes in plasma lipoproteins in patients with diabetes. In type 2 diabetes, the obesity or insulin-resistant metabolic disarray that is at the root of this form of diabetes could, itself, lead to lipid abnormalities exclusive of hyperglycemia.

The lipoprotein abnormalities commonly present in type 2 diabetes, previously termed noninsulin-dependent diabetes mellitus, include hypertriglyceridemia and reduced plasma HDL cholesterol. In addition, low density lipoprotein (LDL) are converted to smaller, perhaps more atherogenic, lipoproteins termed small dense LDL.[40] Moreover, this dyslipidemia often is found in prediabetics, that is patients with insulin resistance but normal indexes of plasma glucose.[41] Therefore, abnormalities in insulin action and not hyperglycemia per se are associated with this lipid abnormality. In support of this hypothesis, some thiazoladinediones improve insulin actions on peripheral tissues and lead to a greater improvement in lipid profiles than seen with other glucose-reducing agents.[42]

A number of studies using tracer kinetics in humans have demonstrated that liver production of apolipoprotein B (apo B), the major protein component of very low density lipoprotein (VLDL) and LDL, is increased in type 2 diabetes.[43] ApoB is a large (more than 500-kDa) protein whose production is not modulated at the level of protein synthesis.. The ensuing increase in fatty acid transport to the liver, which is a common abnormality seen in insulin-resistant diabetes, may cause an increase in VLDL secretion. Tissue culture,[44] animal experiments,[45] and human studies[46] suggest that fatty acids modulate liver Apo B secretion.

Lipoprotein lipase (LpL) is the major enzyme responsible for conversion of lipoprotein triglyceride into free fatty acids. This protein has an unusual intercellular transport. LpL is synthesized primarily by adipocytes and myocytes, but must be transferred to the luminal side of capillary endothelial cells, where it can interact with circulating triglyceride-rich lipoproteins such as VLDL and

chylomicrons.[47] Humans with both type 1 and type 2 diabetes have been reported to have reduced LpL activity measured in postheparin blood;[48] the enzyme is released from the capillary walls and into the circulation by heparin. Several steps in the production of biologically active LpL may be altered in diabetes, including its cellular production[49,50] and possibly its transport to and association with endothelial cells[51] LpL is stimulated by acute[52] and chronic insulin therapy.[53] LpL activity is low in patients with diabetes and is increased with insulin therapy.[53]

Specific lipoprotein abnormalities in diabetes

Postprandial lipemia.

Compared with normal subjects, patients with type 2 diabetes have a slower clearance of chylomicrons from the blood after dietary fat.[48,54,55]

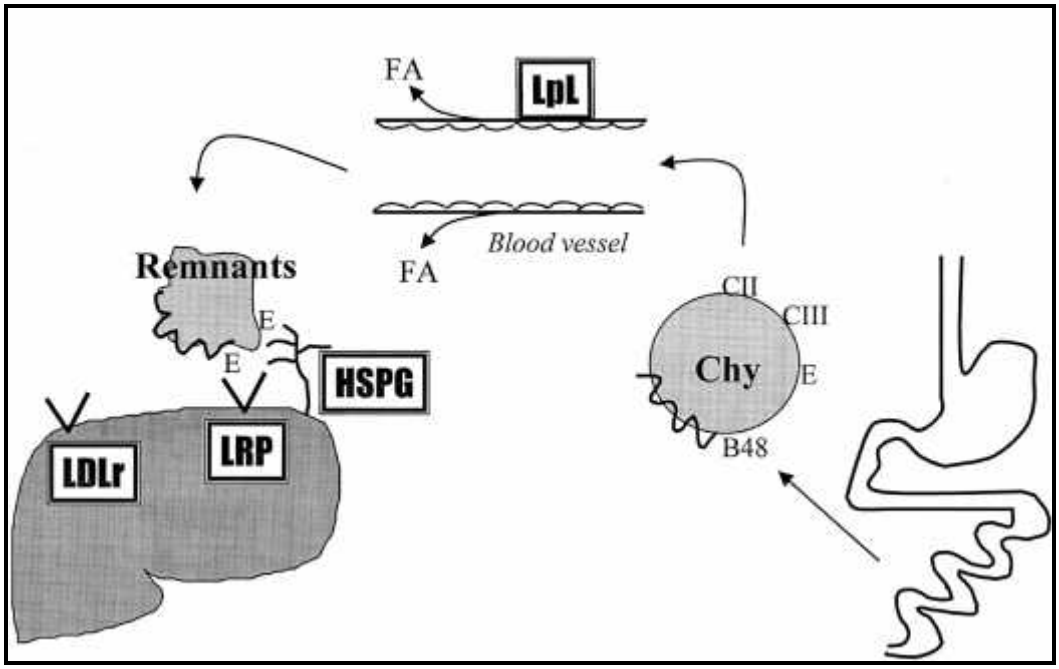


Figure No. 7: Effects of diabetes on postprandial lipemia.

A defect in removal of lipids from the bloodstream after a meal is common in patients with diabetes. Chylomicron metabolism requires that these lipoproteins obtain apo chylomicron II (apo CII) after they enter the bloodstream from the thoracic duct. Triglyceride within the particles can then be hydrolyzed by LpL, which is found on the wall of capillaries. LpL activity is regulated by insulin, and its actions are decreased in diabetes. Triglyceride-depleted remnant lipoproteins are primarily degraded in the liver. This requires them to be trapped by liver heparin sulfate proteoglycans (HSPG) and then internalized by lipoprotein receptors, LDL receptor and lipoprotein receptor protein (LRP). Because remnants contain a truncated form of Apo B, Apo B-48, that does not interact with these receptors, this uptake is mediated by Apo-E. A correlation between postprandial lipemia and atherosclerosis has been found in a number of clinical studies.[56] In addition, Apo B48 remnants are found in a number of atherogenic animal models made with diets and genetic modifications.[57,58] It is generally accepted that remnant lipoproteins, in addition to LDL, are atherogenic.

Remnant lipoproteins can be removed from the bloodstream via several pathways, some of which appear to be modulated by diabetes. Liver is the major, although not exclusive, site of remnant clearance. As these particles percolate through the liver, they are trapped by association with the negatively charged proteoglycans within the space of Disse. This process may be aided by the presence of apoE and hepatic lipase, proteins that bind to both lipid particles and proteoglycans. Both hepatic lipase and heparan sulfate proteoglycan production[59] may be reduced in diabetes. The second step in remnant clearance is via cellular internalization and degradation of the particles. Some of the remnants may be

directly internalized along with cell surface proteoglycans. Most remnant uptake is via receptors. Apo-E is a ligand for both the LDL receptor and LRP. Lipase enzymes (LpL and hepatic lipase) also interact with the LRP. In very poorly controlled diabetes, LDL receptors may be decreased. Although LRP may be regulated by insulin in cultured macrophages,[60] liver LRP is not decreased in diabetic mice.[59]

Effect of increased triglycerides in diabetes mellitus

Although most patients with poorly controlled diabetes develop hypertriglyceridemia, occasional patients develop severe hyperchylomicronemia. Triglyceride levels exceeding 1000 mg/dL lead to visibly lipemic serum. At higher levels the patients can develop eruptive xanthomas, lipemia retinalis, and pancreatitis. Most of these patients have an underlying lipid disorder, such as heterozygous LpL deficiency, that is then exacerbated by diabetes.[61] Elevated plasma triglyceride levels have been shown to be a risk factor for CHD in patients with diabetes.

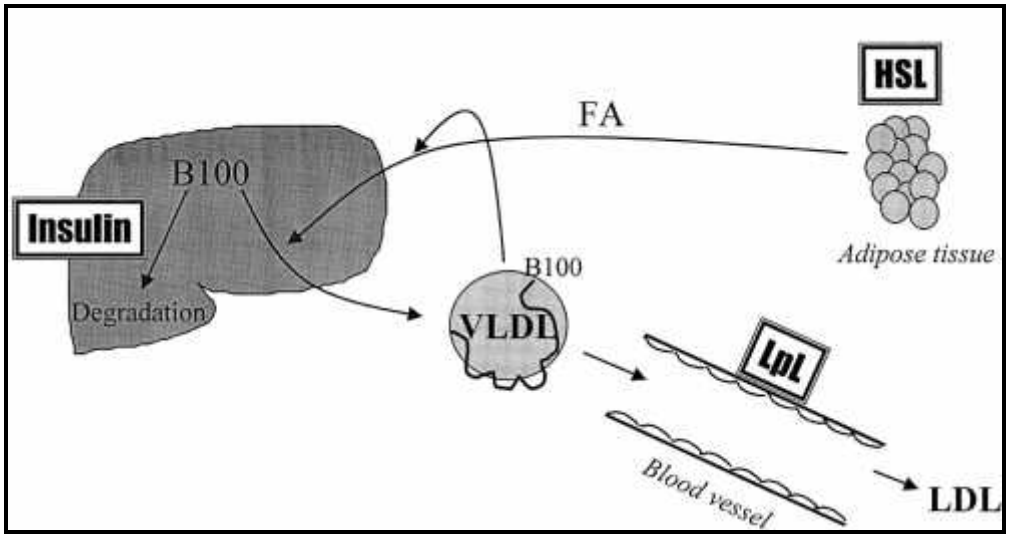


Figure No. 8: Effects of diabetes on very low density lipoprotein production.

Poorly controlled type 2 diabetes is associated with increased plasma levels of VLDL. Overproduction of VLDL may also be caused by obesity. Thus, VLDL metabolism is a competition between liver uptake of partially catabolized lipoproteins and intracapillary lipolysis, a process that may require several steps to complete VLDL conversion to LDL.

Effects of low density lipoprotein in diabetes mellitus

Low density lipoprotein are not usually increased in diabetes. In part this may represent a balance of factors that affect LDL production and catabolism. A necessary step in LDL production is hydrolysis of its precursor VLDL by LpL. A reduction in this step due to LpL deficiency or excess surface apoproteins (C1, C3, or possibly E) decreases LDL synthesis. Conversely, increases in this lipolytic step that accompany weight loss, fibric acid drug therapy, and treatment of diabetes may increase LDL levels. In diabetes a reduction in LDL production may be counterbalanced by decreases in LDL receptors or the affinity of LDL for those receptors. Both glycosylated LDL and small, dense LDL bind to LDL receptors less avidly than does normal LDL. Occasionally diabetic patients, especially those with very poor glycemic control, may have increased LDL that is reduced by treatment of their diabetes. This is due to effects on either the LDL or the receptor.

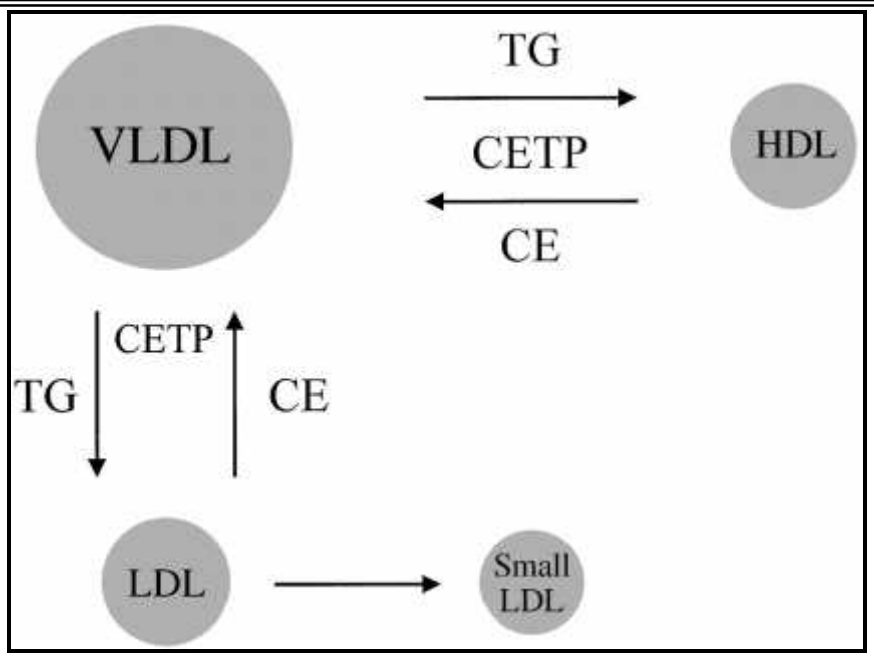


Figure No. 9: Plasma lipid exchange.

A decrease in the size and an increase in density of LDL are characteristic of most hypertriglyceridemic states, including diabetes. Because of this, small dense LDL is considered by many to be one of the hallmarks of diabetic dyslipidemia rather than the expected companion of reduced HDL and increased triglyceride levels.[40] The special designation given to LDL size, rather than HDL and VLDL size, is based on a large amount of clinical and experimental data implying that these particles confer additional atherosclerotic risk. In vitro, small dense LDL can be oxidized more easily, the particles do not interact with LDL receptors as well, and they may associate with proteoglycans on the surface of cells or in matrix more readily. Although several human studies imply that small dense LDL are an additional marker for atherosclerosis development,[62] this observation may be restricted to patients with increased levels of apoB and decreased HDL.[63] In other studies the concomitant association of hypertriglyceridemia and low HDL appears to obscure any additional risk

profiling attributable to LDL size.[64] In dietary studies using primates, larger, not smaller, LDL size correlates with atherosclerosis, presumably because each of these LDL carries more cholesterol.[65]

Effects of high density lipoprotein in diabetes mellitus

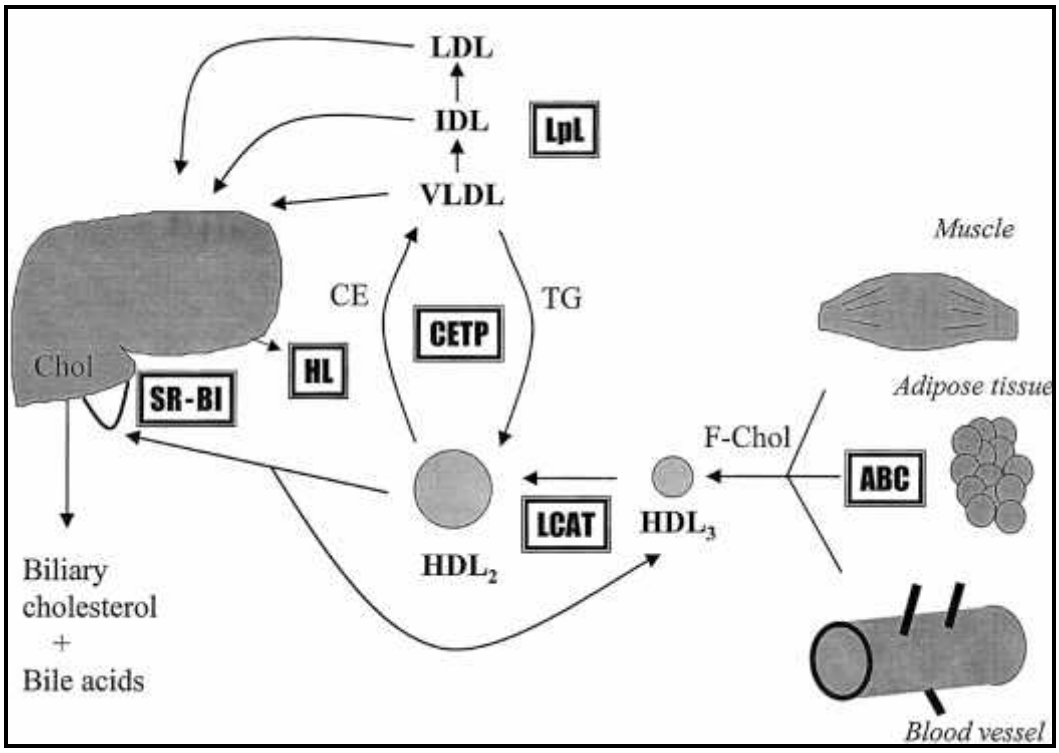


Figure No. 10: Effects of diabetes on high density lipoprotein metabolism.

High density lipoprotein production requires the addition of lipid to small nascent particles. This lipid arrives via hydrolysis of VLDL and chylomicrons with transfer of surface lipids [phospholipid (PL) and free cholesterol (FC)] via the actions of phospholipid transfer protein (PLTP). A second pathway is via efflux of cellular free cholesterol (FC), a process that involves the newly described ATP-binding cassette (ABC1) transporter and esterification of this cholesterol by the enzyme lecithin cholesterol acyl transferase (LCAT). HDL

catabolism may occur through several steps. Hepatic lipase and scavenger receptor-BI are found in the liver and in steroid-producing cells. HDL lipid can be obtained by these tissues without degradation of entire HDL molecules. In contrast, the kidney degrades HDL protein (apoAI) without lipid, perhaps by filtering non lipid-containing protein.

Within the last two year a number of additional enzymes and receptors have been discovered that are integral regulators of HDL metabolism and presumably the effects of HDL on atherosclerosis. It is not yet clear whether hyperglycemia or insulin is an important regulator of these molecules. One of the first steps in HDL production is the addition of lipid to the small, newly formed HDL particles manufactured in the liver and intestine. Phospholipid transfer protein may be required for lipid transfer from triglyceride-rich lipoproteins.[66] In addition, newly formed HDL receive cholesterol from nonhepatic tissues. Theoretically, the most important of these tissues for atherosclerosis development should be the arterial wall and lipid-rich vessel macrophages. Several groups have recently identified the gene responsible for Tangier disease, a rare defect associated with very low levels of HDL and deposits of cholesterol in the tonsils and other lymphoid tissues. ABC1 is defective in this disease.[67] This protein appears to be necessary for transfer of excess cholesterol out of cells and into HDL. Cholesterol is an amphipathic molecule that would be expected to remain on the surface of a lipoprotein. Lecithin acyl transferase converts cholesterol into its hydrophobic ester form, allowing it to enter the core of the lipoprotein particle.

Unlike LDL, but more akin to triglyceride-rich lipoproteins, HDL protein and lipid metabolism are sometimes disparate. Cholesterol is the substrate for

steroid hormones and bile. Liver, adrenal, and gonads can obtain HDL lipid without uptake and degradation of the entire lipoprotein. This process involves scavenger receptor-BI (SRB). By controlling the return of cholesterol to the liver, this receptor appears to play an antiatherogenic role in models of mouse atherosclerosis.[68,69] Kidneys are a major site of degradation of apo AI, the major protein component of HDL. This appears to occur due to filtration of this 22-kDa protein when it is freed from HDL lipid. Fatty acids may be important for this effect, these fatty acids may be derived from hepatic lipase hydrolysis of HDL triglyceride.[70]

Relationship of glycemic control with microvascular and macrovascular complications in type 2 diabetes mellitus.

Trials of glucose reduction have confirmed that glucose control is the key to preventing microvascular diabetic complications. These trials have, however, failed to show a marked benefit of glucose control on macrovascular disease. There are several reasons why this could have occurred. The time course of the effects of diabetes on diseases of large arteries and small vessels differs,[70] and longer trials may be needed. Reversal of underlying vascular disease may require a different degree of control or may follow a different time course than that for small vessels. Finally, the pathological processes are probably different. Small vessel disease of diabetic patients occurs in both type 1 and type 2 diabetes and does not occur in nondiabetics. It is clearly related to the defective glucose control. Large vessel atherosclerosis is not a diabetes-specific disorder, yet it is worse in patients with diabetes. However, processes unrelated to diabetes must be the most important cause. For this reason it may not be surprising that treatment

of these other processes, such as hypertension[71,72] and hyperlipidemia,[73] appears to impact macrovascular disease more than does glucose control. Similarly, the incidence of coronary heart disease in a diabetic population with low plasma cholesterol levels is much less than that found in western, atherosclerosis-prone populations.[74] In contrast, the metabolic abnormalities associated with the insulin-resistant syndrome and increased coronary artery disease are found in the U.S. population even before the development of overt hyperglycemia.[41] Is it these abnormalities and not the glucose per se that are atherogenic ? The answers are yet to be found.

Dyslipidemia and coronary artery disease with reference to lipoprotein (a)

In numerous case-control studies conducted during the past three decades, plasma Lp (a) levels have been determined in patients with established coronary artery disease and compared with those in control subjects. In essentially all such studies, Lp (a) has been demonstrated to be elevated in patients with coronary artery disease. It is possible that Lp (a) may be a marker for coronary artery disease or that Lp (a) levels may increase as a result of coronary artery disease. However, no direct evidence supports the hypothesis that plasma Lp (a) levels increase with the development of atherosclerosis.

The role of elevated Lp (a) concentrations as a risk factor for coronary artery disease in women has not been thoroughly examined. The most interesting results have been obtained in studies of black and white subjects. In the Bogalusa Heart Study Lp (a) concentrations in white and black children and the incidence of myocardial infarction in their parents were used as a measure of future risk for

the children.[75] Lp (a) concentrations in white children with parental myocardial infarction were significantly higher than in white children without parental myocardial infarction.[75]

In addition, the prevalence of parental myocardial infarction in whites was significantly higher in subjects with Lp (a) concentrations more than 25 mg/dL. No relation between Lp (a) concentration and incidence of parental myocardial infarction was observed in blacks. Other investigators have reported that although Lp (a) concentrations in blacks averaged at least twice those observed in whites, the incidence of coronary artery disease was not different between the two racial groups. These findings have prompted several investigators to speculate that the atherogenicity of Lp (a) in blacks is decreased or perhaps counterbalanced by other factors. It has recently been hypothesized that in blacks, the lower atherogenicity of Lp (a) may be explained by the fact that fewer blacks than whites have the combination of high Lp (a) levels and small Apo (a) sizes In the range of small Apo (a) isoforms, there is no significant difference in Lp (a) values between blacks and whites. These findings prompted the investigators to speculate that high Lp (a) values associated with small Apo (a) isoforms may be more atherogenic than elevated values associated with medium or large Apo (a) isoforms. This hypothesis is intriguing and the predictive value of small Apo (a) isoform sizes and high Lp (a) levels in coronary artery disease clearly warrants further investigation in prospective studies.

It is evident that if Lp (a) concentration is above 30 mg/dL, as it is about 20% of Caucasians, the relative risk of coronary atherosclerosis rises two fold.

However, when LDL and Lp (a) concentration both are elevated the relative risk is above five fold.[9]

The association between elevated levels of Lp (a) and CHD appears stronger in men under 56 years of age and is responsible for much of the familial predisposition observed in individuals.[9]

A study of Lp (a) concentration in Tuniscan type 2 diabetic patients with relationship to glycemic control of CHD found diabetic patients with and without CHD had significantly higher levels of Lp (a). A correlation was observed between Lp (a) levels of total cholesterol in all diabetic patients particularly in diabetic men. Male patients with CHD showed significant higher Lp (a) than those without CHD. Elevated levels of Lp (a) and abnormal lipid profile in diabetic men suggest their involvement in atherogenesis and subsequent development of CHD.[76]

The last 25 years have witnessed an exponential increase of interest in the lipoprotein Lp (a) the structure of the gene encoding for its unique apoprotein.

Diabetes is a state of increased free radical activity. Lipid peroxidation of cellular structures, a consequence of free radical activity, is thought to play an important role in aging, atherosclerosis and late diabetic complications.

Diabetes is a syndrome which consists of metabolic and vascular component consisting of derangements of carbohydrate, fat and protein with hyperglycemia and glycosuria secondary to either an absolute or relative deficiency of insulin. The vascular component consists of accelerated atherosclerosis with microaneurysm formation and neovascularisation.

Dyslipidemia is a relatively common problem in patients with poorly controlled diabetes. Since both diabetes and dyslipidemia are considered to be major risk factors for the development of premature atherosclerosis, it is hoped that control of both of these factors with appropriate treatment will lower the risk of heart disease and microvascular complications in diabetic patients.

In known diabetics, both juvenile and adult onset types there is atleast a two fold increase in incidence of myocardial infarction compared with non diabetics.[9] This risk is markedly increased in younger diabetics and diabetic women are more prone to IHD than diabetic men.

Except niacin and hormone replacement therapy, no other lipid lowering agent lowers Lp (a) levels. Other drugs such as cyclosporin, danazol, and stanazolol can increase the Lp (a) levels.

Lipoprotein (a) is also found to be elevated in other microvascular complications of DM. Since the pathogenesis of atherosclerosis and microangiopathy remains obscure, a close clinical evaluation of serum lipids and lipoprotein profile in diabetics continues to be important.

ROLE OF LIPOPROTEIN (A) IN OTHER MICROVASCULAR COMPLICATIONS OF DIABETES MELLITUS

Diabetic retinopathy

Diabetic retinopathy is the most frequent cause of blindness among adults aged 20 to 74 years. During the first two decades of disease, nearly all patients with type 1 DM and more than 60% with type 2 DM have retinopathy. In type 2 DM, 21% of patients have retinopathy at first diagnosis.

Classification (Modified from American Academy of Ophthalmology)[77]

Non Proliferative Diabetic Retinopathy (NPDR)

1. ***Mild NPDR***

   At least one retinal microaneurysm and one or more of the following : retinal hemorrhage, hard exudates, soft exudates.

2. ***Moderate NPDR***

   Hemorrhages or microaneurysms or both in atleast one quadrant and one or more of the following: soft exudates, venous beading and IRMA.

3. ***Severe NPDR***

   Hemorrhages or microaneurysms or both in all quadrants, venous beading in two or more quadrants, IRMA in at least one quadrant.

Proliferative Diabetic Retinopathy (PDR)

1. ***Early PDR***

One or more of the following:

- NVE
- NVD
- Vitreous or preretinal hemorrhage.
- NVE less than half disc area.

2. ***High risk PDR***

One or more of the following.

- NVD more than one fourth to one third disc area.
- NVD with vitreous or preretinal hemorrhage.
- NVE > ½ disc area. Preretinal or vitreous hemorrhage.

3. ***Advanced PDR***

High risk PDR, traction retinal detachment involving macula or vitreous hemorrhage obscuring ability to grade NVD or NVE.

- Intraretinal microvascular abnormalities (IRMA).
- Neovascularisation elsewhere (NVE).
- Neovascularisation disc (NVD).

Diabetic retinopathy progresses from mild non-proliferative abnormalities to moderate and severe non-proliferative diabetic retinopathy to proliferative

diabetic retinopathy. Macular edema can develop at all stages of diabetic retinopathy. NPDR usually develops late in first decade or early second decade of type 2 DM. PDR usually develops within five years of NPDR. Pregnancy, poor glycemic control, hypertension and cataract surgery accelerate these changes. UKPDS study revealed that for every percentage reduction of HbA1C (for example from seven to eight percent), there was a 35% reduction in risk of retinopathy, and tight blood pressure control (to less than 150/85 mm Hg) results in 34% reduction in progression of retinopathy.[73]

Proliferative diabetic retinopathy (PDR) is the most common cause of acquired blindness in adults. Capillary occlusion is a frequent finding in diabetic retinopathy and is believed to play an important role in the development of PDR. Regarding serum Lp (a) levels in PDR, studies reported that serum Apo (a) levels were raised in active diabetic retinopathy groups (severe NPDR or PDR) compared with no diabetic retinopathy group.[78] Another study also reported higher prevalence of pre proliferative retinopathy with increasing Lp (a) levels in type 2 diabetic patients. Serum Lp (a) levels were increased in patients with overt proteinuria.[4] The mechanisms of association between PDR and high Lp (a) levels remain to be elucidated. Lp (a) is believed to have an antifibrinolytic effect, so it may contribute to the occlusion of small retinal vessels and, therefore, it may be an independent risk factor for PDR. Another study showed that retinal blood flow progressively decreases from the very early stage of diabetic retinopathy, reflecting increasing resistance to flow through the retinal vascular network.[81] This decrease is followed by a paradoxical increase in retinal blood flow, which was attributed to formation of shunts between arterioles and venules in the areas

of capillary occlusion. Thus increased serum Lp (a) may contribute to the increased resistance to flow or capillary occlusion in the development or progression of diabetic retinopathy.

In diabetes, conflicting reports are available regarding prognostic significance of Lp (a) levels.[79] A few studies record that it may be elevated in insulin-dependent DM. Particularly, patients with microalbuminuria and proliferative retinopathy show higher Lp (a) levels.[80] Similarly, Lp (a) has been correlated to CAD in diabetics in some studies, while other trials do not show any such correlation. South Indian non-insulin-dependent DM (NIDDM) patients with high Lp (a) levels, however, show good correlation with CAD.[71]

DIABETIC NEPHROPATHY

Diabetes has become the most common single cause of end stage renal disease (ESRD) world wide. About 20 to 30% of patients with type 1 or type 2 DM develop evidence of nephropathy, but in type 2 diabetes a considerably smaller fraction of these progress to ESRD. However, because of much higher prevalence of type 2 DM, these patients constitute over half of patients with nephropathy needing dialysis.[81]

The diabetic nephropathy progresses from appearance of low but abnormal levels of (more than or equal to 30 mg to 299 mg/day or 20µg/min) albumin in urine (stage of microalbuminuria) to stage of macroalbuminuria or clinical albuminuria (more than or equal 300 mg/dL or more than or equal 200 µg/min) to ESRD. Progress from microalbuminuria to macroalbuminuria usually takes 10 to 15 yeas. ESRD develops in 50% of type 1 diabetic individuals with

clinical nephropathy within 10 years and in 75% by 20 years. But in type 2 DM, even after 20 years of overt nephropathy only 20% progress to ESRD.

A test for the presence of urinary microalbumin should be performed at diagnosis in patients with type 2 DM and after five years of disease duration in those with type 1 DM, then repeated annually. Screening for microalbuminuria can be performed by three methods.

1. Measurement of the albumin to creatinine ratio in a random spot urine collection.
2. 24 hour urine collection and measurement of albumin excretion.
3. Timed (e.g. four hour or overnight) collection.

24 hour collection of urine is most reliable.

Table No. 2: Definitions of abnormalities in albumin excretion

CATEGORY	Spot collection (µg/mg creatinine)	24 Hr collection (mg/24 hours)	Timed collection (µg/min)
Normal	< 30	< 30	< 20
Microalbuminuria	30 to 299	30 to 299	20 to 199
Clinical albuminuria	≥ 300	≥ 300	≥ 200

(Adapted from ADA, 2004)

In addition to being the earliest manifestation of nephropathy, albuminuria is a marker of greatly increased cardiovascular morbidity and mortality for patients with either form of diabetes.[82]

A prospective study of Lp (a) as a risk factor for deteriorating renal function in type 2 DM patients with overt proteinuria substantiated that Lp (a) is an independent risk factor for the progression of diabetic nephropathy in type 2 diabetic patients.[4]

DIABETIC NEUROPATHY

Diabetic neuropathy occurs in approximately 50% of individuals with long standing type 1 or type 2 DM. As with other complications of DM, the development of neuropathy correlates with the duration of diabetes and glycemic control.

Classification of diabetic neuropathy

Symmetric

1. Distal, primarily sensory polyneuropathy.
2. Autonomic neuropathy
3. Chronic proximal motor neuropathy

Asymmetric

1. Acute or subacute proximal motor neuropathy.
2. Cranial mononeuropathy
3. Truncal neuropathy
4. Entrapment neuropathies

Also classified as follows (Watkin's and Edmond's classification)[83]

1. Progressive Neuropathies

- Chronic sensory motor neuropathy
- Autonomic neuropathy

2. Reversible Neuropathies

- Mononeuropathies
    - Proximal motor neuropathy (Amyotrophy)
    - Cranial nerve palsies (III,IV,VI)
    - Truncal radiculopathies
- Acute painful neuropathies

3. Pressure Palsies

- Carpal tunnel syndrome.

The most common form of diabetic neuropathy is distal symmetric polyneuropathy. Most frequently presents with distal sensory loss. Hyperesthesia, paresthesia and dysthesia can also occur.

Painful neuropathies may develop in these patients. Both an acute (lasting less than 12 months) and a chronic form of painful diabetic neuropathy have been described. Individuals with long standing type 1 or type 2 DM may develop autonomic neuropathy. This can involve multiple systems, including the cardiovascular, gastrointestinal, genitourinary and metabolic systems. Resting tachycardia, orthostatic hypotension, gastroparesis, bladder emptying abnormalities, hyperhydrosis of upper extremities and anhydrosis of lower extremities are features of diabetic autonomic neuropathy.

Lipoprotein (a) and peripheral arterial disease

A study conducted to examine the association between Lp (a) and peripheral arterial disease (PAD) and determine the optimal cutoff in Chinese type 2 diabetic patients in Taiwan showed that, Lp (a) is a significant and independent risk factor for PAD in type 2 diabetic patients in Taiwan. It is also significantly predictive for the severity of PAD, especially in patients with existent PAD or an ABI 0.9. The optimal cutoff of Lp (a) for PAD in type 2 diabetic patients in Taiwan is 13.3 mg/dL, a level much lower than the commonly recommended 30.0 mg/dL in Caucasians.[84]

Lipoprotein (a) is now established as a genetically determined predictor of atherosclerotic vascular diseases and in particular premature CAD. High levels of this lipoprotein, particularly in Asian Indians is a matter of clinical concern. Since it is not generally amenable to the lifestyle measures, other lipid and non-lipid risk factors must be modified to decrease the risk in diabetic patients with high Lp (a) levels.

METHODOLOGY

The present study was conducted in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum on patients with type 2 DM during the period of January 2007 to December 2007.

Study design

One year correlation study.

Study period

The present study was conducted during January 2007 to December 2007.

Method of collection of data

Source of Data

Patients diagnosed to have type 2 DM more than 10 years proved by laboratory reports at KLES Dr. Prabhakar Kore Hospital and MRC Belgaum were selected for the study.

Sample size

A sample size of 50 cases was with type 2 DM were selected for the study.

Sampling procedure

A sample size of 50 cases with type 2 DM was calculated assuming the mean Lp (a) in the sample to be 30 and the population to be 31 also considering

sample standard deviation of two, the minimum sample size was calculated to be 31 with $\alpha = 0.05$ and $\beta=0.80$.

Selection criteria

Inclusion Criteria

- Patient diagnosed to have Type II Diabetes more than 10 years proved by laboratory reports satisfying WHO criteria : FBS more than or equal to 126 mgdl and PPBS more than or equal to 200 mg/dl.

Exclusion Criteria

- All patients with documented liver disorder.
- All patients on hypolipidemic drugs.
- All patients with documented chronic kidney disease.
- All patients with documented coronary artery disease.

Procedure

Patients attending to the out patients department of Medicine at KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum diagnosed to have type 2 DM more than 10 years proved by laboratory reports were evaluated and selected by detailed medical history and physical examination. The study was approved by the Ethical and Research Committee of J. N. Medical College, Belgaum.

After finding the suitability as per inclusion and exclusion criteria they were selected for the study and briefed about the nature of the study, the

interventions used and written informed consent was obtained (Annexure–I). Further, descriptive data of the participants like name, age, sex, detailed history, were obtained by interviewing the participants and clinical examination and necessary investigations were recorded on predesigned and pretested proforma (Annexure-II).

Detailed relevant history regarding duration of Type II Diabetes Mellitus and treatment history is taken. Detailed clinical examination, including peripheral pulses for peripheral vascular disease and fundus examination for diabetic retinopathy changes will be done. Fasting and post prandial blood sugars are measured by Hexokinase method.

Fasting lipid profile is measured by following methods:

- Cholesterol – Cholesterol Oxidase method.
- HDL – Accelerator Selective Detergent Methodology.
- LDL- Direct Method.
- TGL – Lipase or Glycerol dehydrogenase method.

Measurement of Lipoprotein (a)

- Randox immunoturbido- metric immunoassay

Other tests

- Complete Blood Count
- Liver Function Test
- Minirenal
- Urine routine

- 24 hrs proteinuria
- ECG

Randox immunoturbido- metric immunoassay

Intended use

For the quantitative in vitro determination of Lipoprotein (a) in human serum or plasma. This product is suitable for use on the Dade Dimension® AR, ES. ARX, XL, RXL, and XPAND analysers. (Cat No. LP 2878)

Clinical significance

The Lipoprotein (a) test system is intended to measure lipoprotein (a) in serum. Lipoprotein (a) measurements are used to evaluate disorders of lipid metabolism and to assess coronary heart disease in specific populations.

Principle

Agglutination occurs due to an antigen-antibody reaction between Lp(a) in a sample and anti-Lp(a) antibody adsorbed to latex particles. This agglutination is detected as an absorbance change at 700 nm proportional to the concentration of Lp(a) in the sample.

Sample collection and storage

Collect serum using standard sampling tubes and plasma using tubes containing Li heparin, Na heparin, Na EDTA, K EDTA or citrate. The samples should be analysed immediately or stored at -20°C or -70°C for delayed testing. Freeze thaw cycling is not recommended.

Reagent composition

Wells		**Initial concentration**
1 – 2	**Lp (a) Latex Reagent**	
	Suspension of latex particles coated with anti-Lp(a) antibodies	0.5%
	Glycine	0.17M
	Sodium chloride	0.1 M
	Sodium azide	≤ 0.09% w/v
3 – 6	**Lp (a) Assay Buffer**	
	Glycine	0.17 M
	Sodium Chloride	1.08 M
	EDTA disodium salt	0.05 M
	Sodium azide	≤ 0.09% w/v

Calculation of results

The instrument automatically calculates and prints the concentration of Lipoprotein (a) in mg/dL using the calculation scheme illustrated in Dimension System manual.

Interference

The following analytes were tested up to the noted levels and did not cause interferences:

Intralipid	5%
Bilirubin	35 mg/dL

Haemoglobin	1040 mg/dL
Ascorbic acid	50 mg/dL
Triglycerides	493 mg/dL
Plasminogen	200 mg/dL
Apolipoprotein B	200 mg/dL

Normal values

Adults less than 30 mg/dL

The above reference range was established based on a sample of 96 Caucasian individuals comprising 49 males (age range 17 to 90 years; mean equal to 55 years) and 47 females (age range 13 to 84 years; mean equal to 55 years) resident in Northern Ireland. The population tested was an ambulatory population with no history of coronary disease. Results showed a mean Lp (a) value of 18.5 mg/dL for males and 20.6 mg/dL for females. Reference ranges have not been established for this assay for different ethnic populations or disease states.

Lp(a) concentrations have been shown to be genetically determined and to vary with ethnic populations. One study carried out in the United States showed that mean plasma levels of Lp (a) were approximately twice as high in African people or people of African descent compared to levels in Caucasians. Also, the distribution of Lp (a) is less skewed in African people or people of African descent than in Caucasians. Other studies have also shown no difference in Lp (a) levels between men (mean = 14 mg/dL) and women (mean = 15 mg/dL).. Levels of Lp (a) have been shown not to differ significantly between pre and post

menopausal Caucasian women.

It is therefore recommended that each laboratory establish its own reference range to reflect the age, race, sex, diet and geographical location of the population.

Assay range

The range of this assay is approximately 4 to 90 mg/dL. If the sample concentration exceeds the assay range, dilute the sample 1 +2 with 0.9% NaCI solution and reassay. Multiply the result by three.

These values are dependent on the lot specific values of the calibrators in use.

Specific performance characteristics

The following performance data were obtained on Dade Dimension AR and RXL analyzers at 3rC.

Linearity

The lipoprotein (a) assay was found to be linear up to 86.58 mg/dL

Sensitivity

The minimum level of lipoprotein (a) detectaple with an acceptable level of precision has been determined as 4.0 mg/dL.

Prozone effects

Antigen excess effects are not noted up to 250 mg/dL.

Statistical analysis

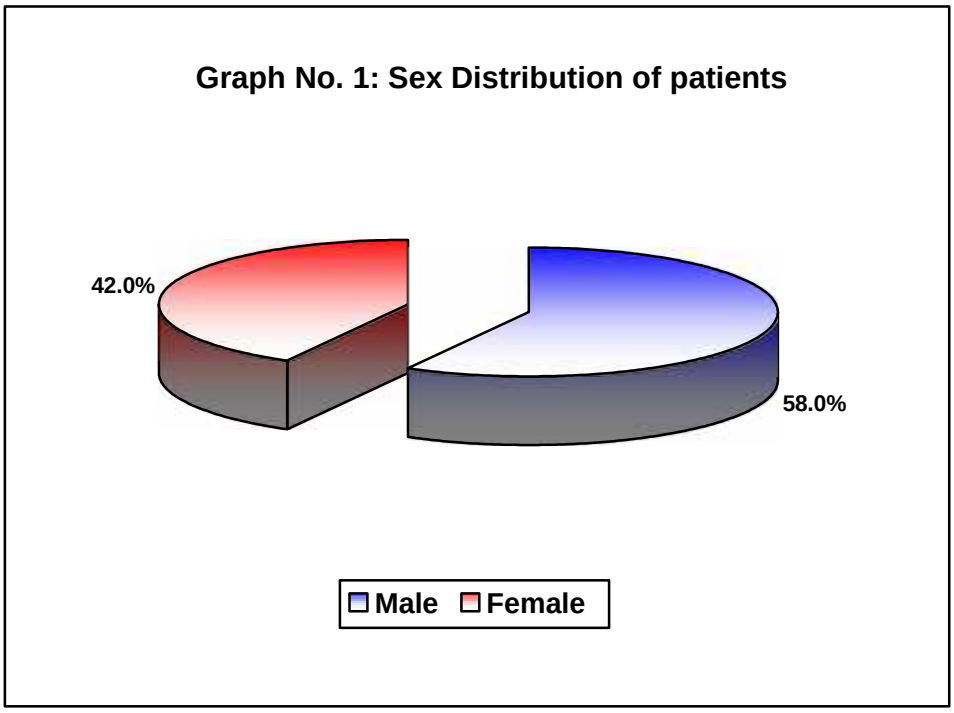
Statistical Analysis will be performed using SPSS statistical package (Chicago IL). The Mean, Standard Deviation, Confidence interval for different parameters associated with different microvascular and macrovascular complications would be calculated. Correlation coefficient between related variables would also be estimated. Differences in continuous variables between groups will be analysed by students 't' test and chi-square test will be used to compare frequencies between different groups.

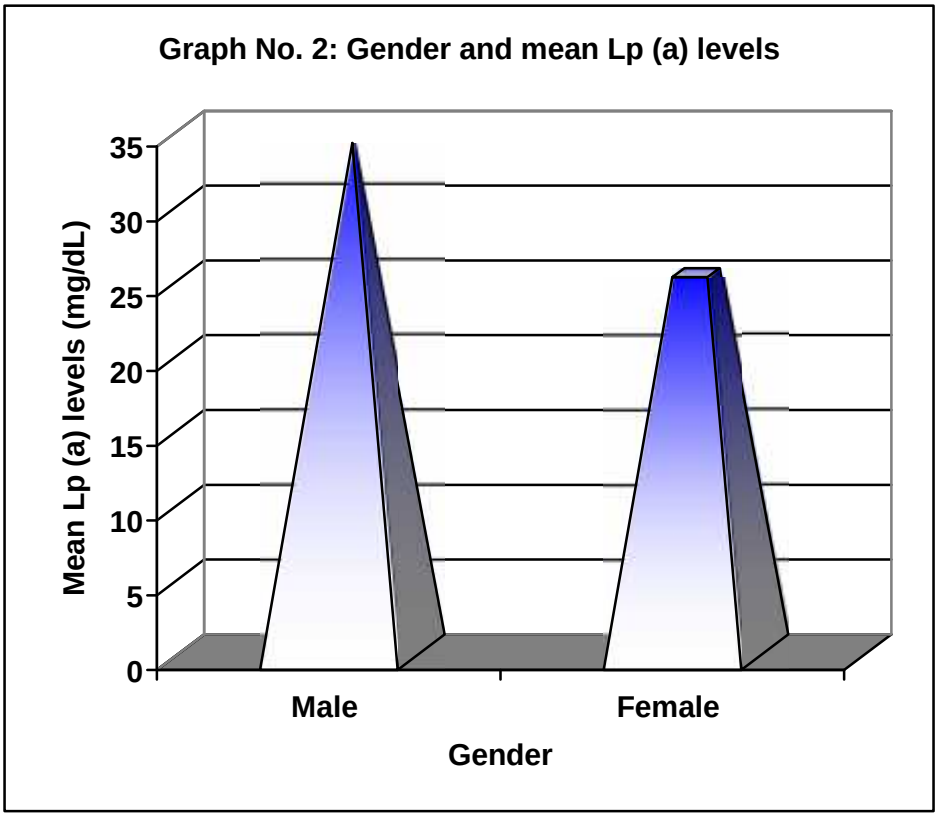
RESULTS

The present correlation study was conducted in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum on 50 patients, detected to have laboratory proven type 2 DM for more than 10 years duration. The serum Lp (a) levels and the association with micro and macro vascular complications of DM were studied for a period of one year during January 2007 to December 2007. The findings are tabulated as below.

Table No. 3: Genderwise distribution patients and mean Lp (a) levels

Gender	Number of patients		Lp (a) levels (mg/dL)	
	Number	**Percentage**	**Mean**	**S.D.**
Male	29	58.0%	34.05	15.67
Female	21	42.0%	25.37	12.26
Total	**50**	**100.0%**	30.40	14.85



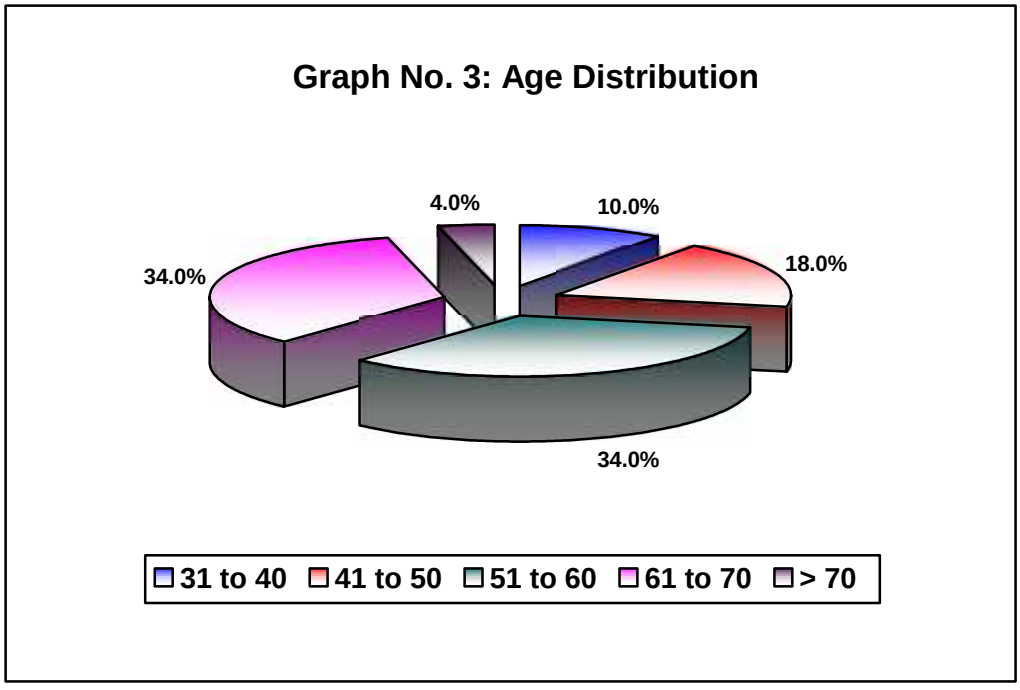


In the present study among 50 patients 29 (58.0%) were males and 21 (42.0%) were females with male to female ratio of 1.38: 1.

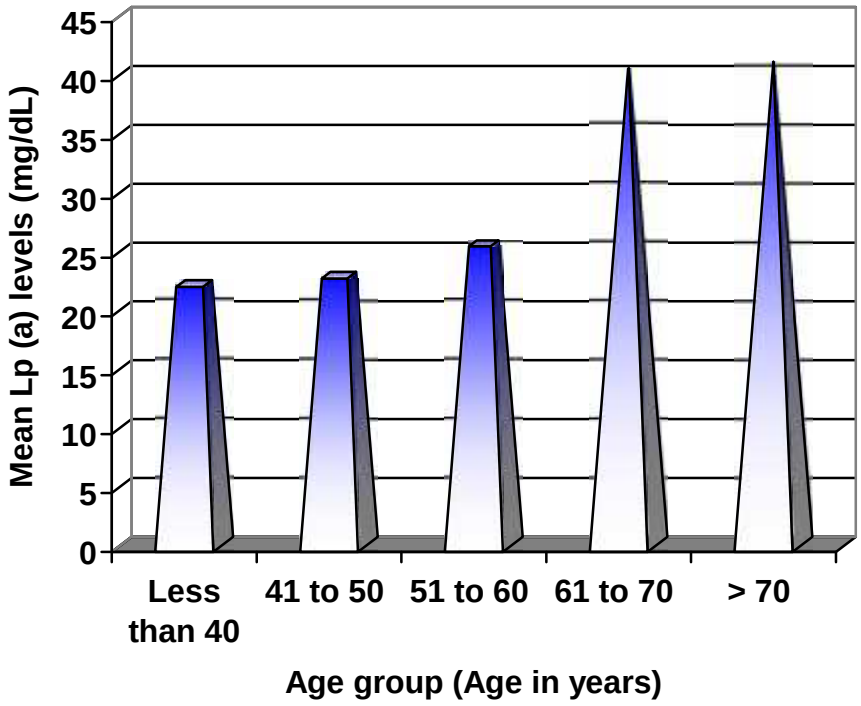
Table No. 4: Age distribution and mean Lp (a) levels

Age group (Age in years)	Number of Patients		Mean Lp (a) levels (mg/dL)	
	Number	Percentage	Mean	S.D.
Less than 40	05	10.0	22.16	6.601
41 to 50	09	18.0	22.87	4.301
51 to 60	17	34.0	25.58	2.235
61 to 70	17	34.0	40.40	4.036
More than 70	02	4.0	41.00	2.666
Total	**50**	**100.0%**	**30.41**	**2.100**

x^2 = 18.8 f = 4.67 **p = 0.011**



Graph No. 4: Comparison of age and mean Lp (a) levels

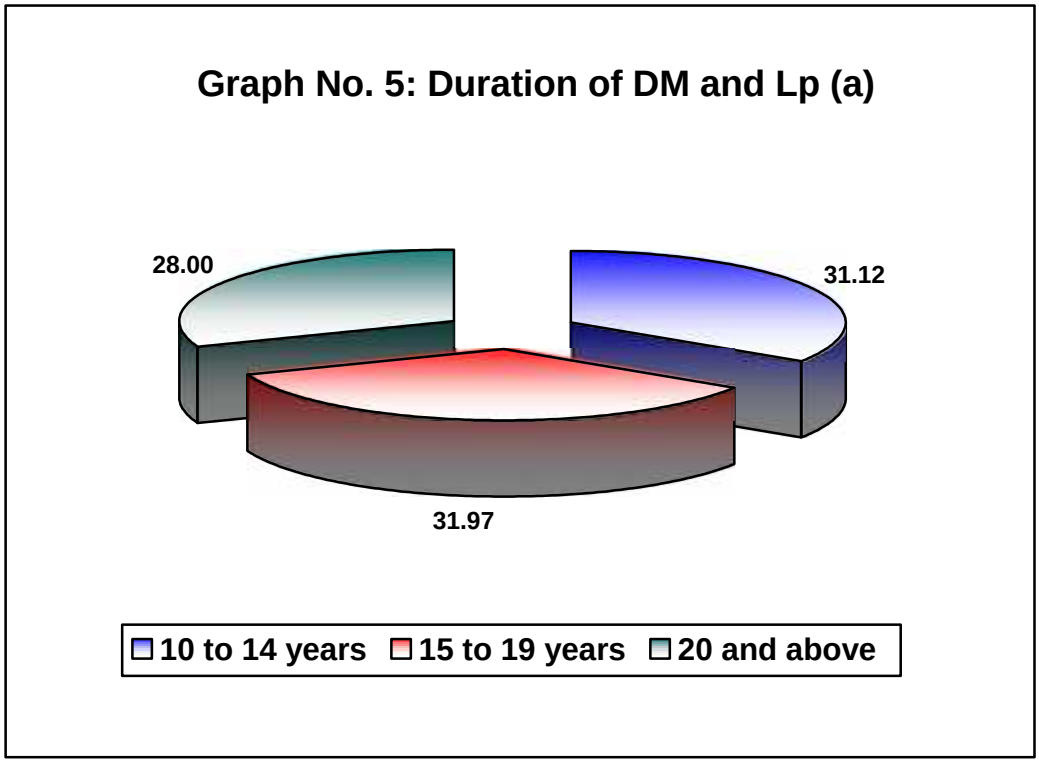


In this study the age of the patients varied from 39 years to 75 years of age. Majority (34.0%) of the patients belonged to 51 to 60 years and 61 to 70 years age group followed by 18.0% in 41 to 50 years age group, 10.0% in less than 40 years age group and 4.0% in more than 70 years age group. The findings revealed that as age progressed serum Lp (a) levels progressively increased significantly (p=0.011).

Table No. 5: Comparison of duration of DM and mean Lp (a)

Duration (In years)	Number of patients		Lp (a) levels	
	Number of	Percentage	Mean	(mg/dL)
10 to 14 years	40	80.0%	31.12	15.96
15 to 19 years	09	18.0%	31.97	10.20
20 and above	01	2.0%	28.00	-
Total	**50**	**100.0%**	**30.41**	**14.85**

x^2 = 50.92 f = 0.068 p = 0.935

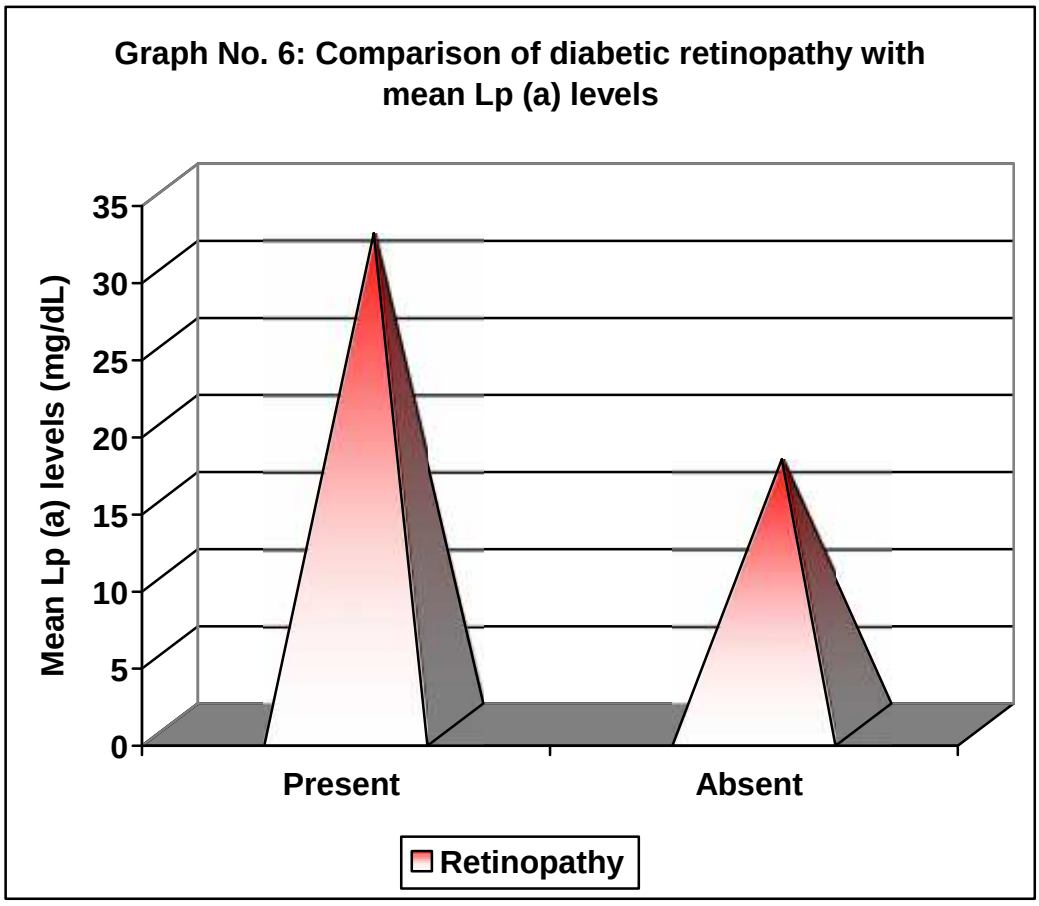


In the present study **m**ajority of the patients had duration of 10 to 14 years (80%) and chi-square test revealed a significant difference between those frequencies. When mean Lp (a) levels were verified against DM duration, it was found that mean Lp (a) levels were independent of duration of DM (p = 0.935).

Micorvascular complications of DM and mean Lp (a) levels.

Table No. 6: Comparison of diabetic retinopathy with mean Lp (a) levels

Complications		Number of patients		Mean Lp (a) levels (mg/dL)		t value	p value
		No.	%	Mean	S.D.		
Retinopathy	Present	17	34.0%	31.87	14.69	2.171	**0.035**
	Absent	33	66.0%	17.22	9.15		
Total		**50**	**100.0%**	**30.40**	**14.85**		



In the present study 34.0% had retinopathy with mean Lp (a) levels of 31.87 mg/dL and 66.0% patients had no retinopathy with mean Lp (a) levels of

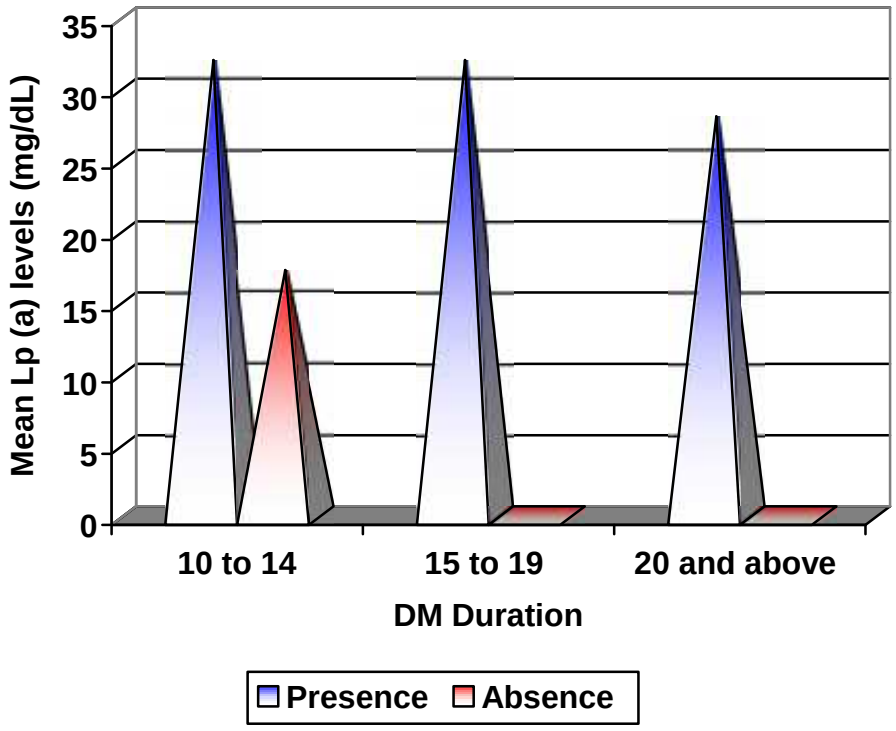
17.22 mg/dL, suggesting that patients with retinopathy had raised serum Lp (a) levels compared with patients without retinopathy (p = 0.035).

Table No. 7: Comparison of DM duration and diabetic retinopathy with mean Lp (a) levels

DM duration (Years)	Retinopathy	Number	Percentage	Mean Lp (a) levels (mg/dL)	S.D.
10 to 14	Present	35	70.0%	31.95	15.95
	Absent	05	10.0%	17.22	9.15
	Total	40	80.0%	30.11	15.96
15 to 19	Present	09	18.0%	31.96	10.20
	Absent	00	0.0%	-	-
	Total	09	18.0%	31.96	10.20
20 and above	Present	01	2.0%	28.00	-
	Absent	00	0.0%	-	-
	Total	01	2.0%	28.00	-
Total	Present	45	90.0%	31.87	14.69
	Absent	05	10.0%	17.22	9.15
	Total	50	100.0%	30.40	14.85

t=2.171 **p= 0.035**

Graph No. 7: Comparison of DM duration and retinopathy with mean Lp (a) levels



In the present study while comparing the duration of diabetes with presence or absence of retinopathy and the mean Lp (a) levels, the results revealed that those who had retinopathy had significantly higher Lp (a) values compared to those who did not have retinopathy and 't' value revealed a significant difference (t=2.171; p= 0.035). There was no statistically significant association between DM duration and mean Lp (a) levels but none of the cases were devoid of retinopathy when the DM duration was more than 15 years.

Table No. 8: Comparison of diabetic nephropathy and mean Lp (a)

Nephropathy (Sr. creatinine levels mg/dL)	Number of patients		Mean Lp (a) levels (mg/dL)	
	Number	Percentage	Mean	S.D.
Less than 1	29	58.0%	29.96	14.01
1 to 1.5	14	28.0%	28.74	10.39
More than 1.5	07	14.0%	35.61	24.71
Total	50	100.0%	30.40	14.85

f = 0.522 p = 0.597

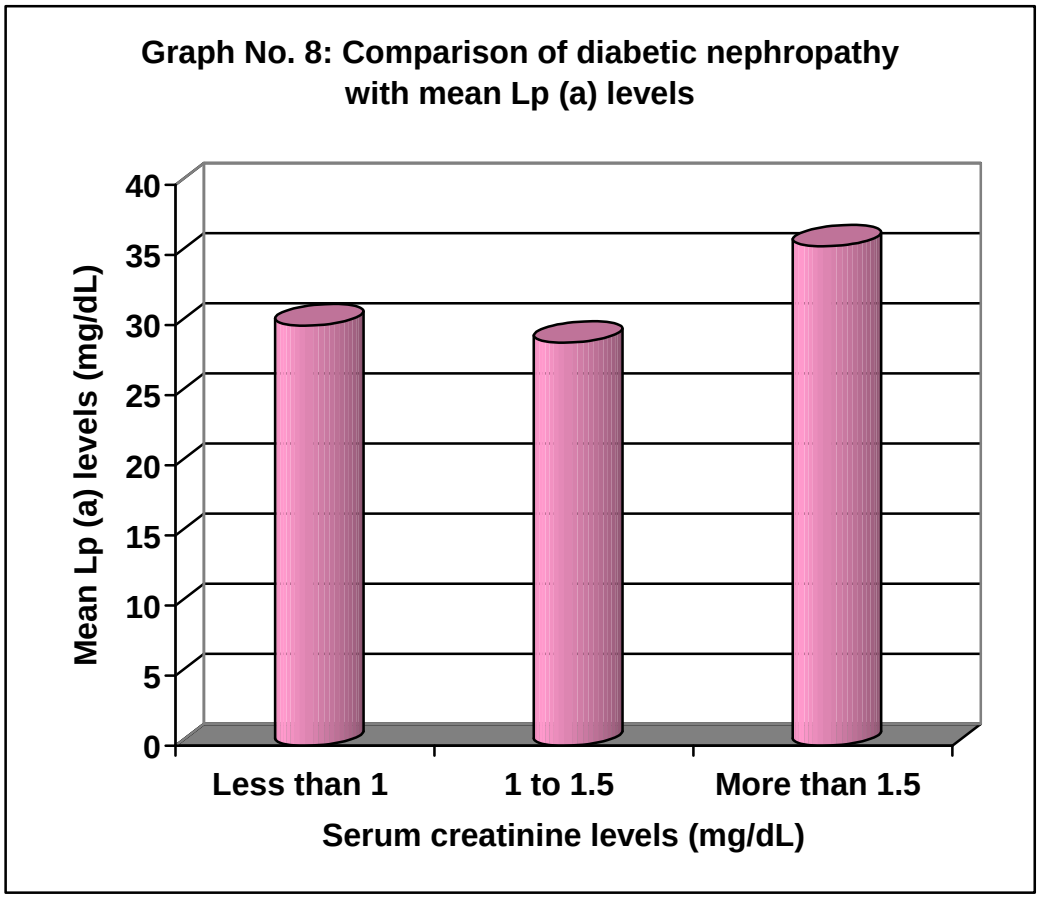
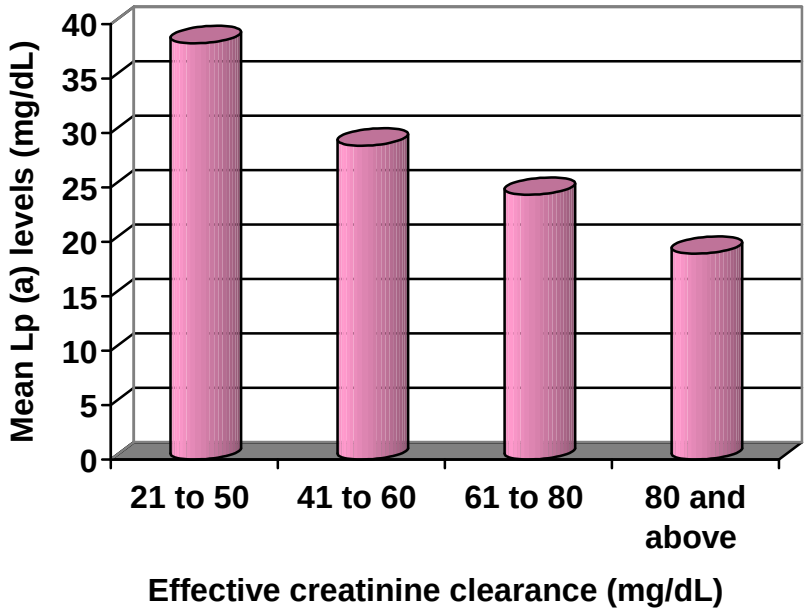


Table No. 9: Comparison of effective creatinine clearance and mean Lp (a) levels

Effective Creatinine clearance (mg/dL)	Number of patients		Lp (a) levels (mg/dL)	
	Number	**Percentage**	**Mean**	**S.D.**
21 to 50	15	30.0%	38.25	19.80
41 to 60	27	54.0%	28.84	10.81
61 to 80	03	6.0%	24.33	12.09
More than 80	05	10.0%	18.92	7.47
Total	**50**	**100.0%**	**30.40**	**14.85**

f = 2.982 **p = 0.040**

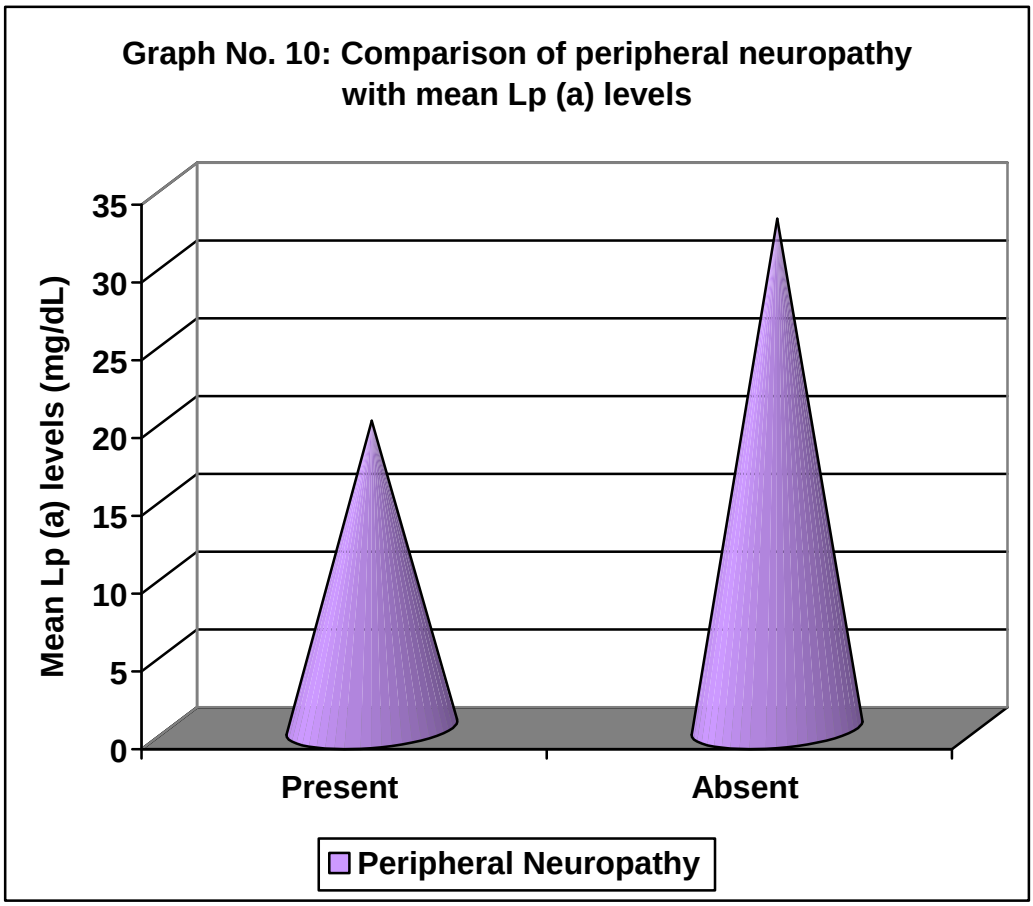
Graph No. 9: Comparison of effective creatinine clearance with mean Lp (a) levels



In the present study of 50 diabetic patients 29 (58.0%) patients had serum creatinine less than 1 mg/dL. Fourteen (28.0%) patients had creatinine levels between 1 to 1.5 mg/dL and seven (14.0%) patients had more than 1.5 mg/dL and these patients had progressively increasing values of mean Lp (a) levels. Patients with serum creatinine > 1.5 mg/dL had maximum mean Lp (a) level of 35.61 mg/dL when compared to mean Lp (a) level of 29.96 mg/dL among patients with serum creatinine less than 1 mg/dL. But it was statistically insignificant. Similarly comparison between creatinine clearance and mean Lp (a) levels showed an inverse relationship that, as creatinine clearance decreased mean Lp (a) levels increased (p = 0.040).

Table No. 10: Comparison of peripheral neuropathy with mean Lp (a) levels

Complications		**Number of patients**		**Mean Lp (a) levels (mg/dL)**		**t value**	**p value**
		Number	**Percentage**	**Mean**	**S.D.**		
Peripheral Neuropathy	Present	41	82.0%	32.74	14.51	2.500	**0.016**
	Absent	09	18.0%	19.76	11.91		
Total		**50**	**100.0%**	**30.40**	**14.85**		

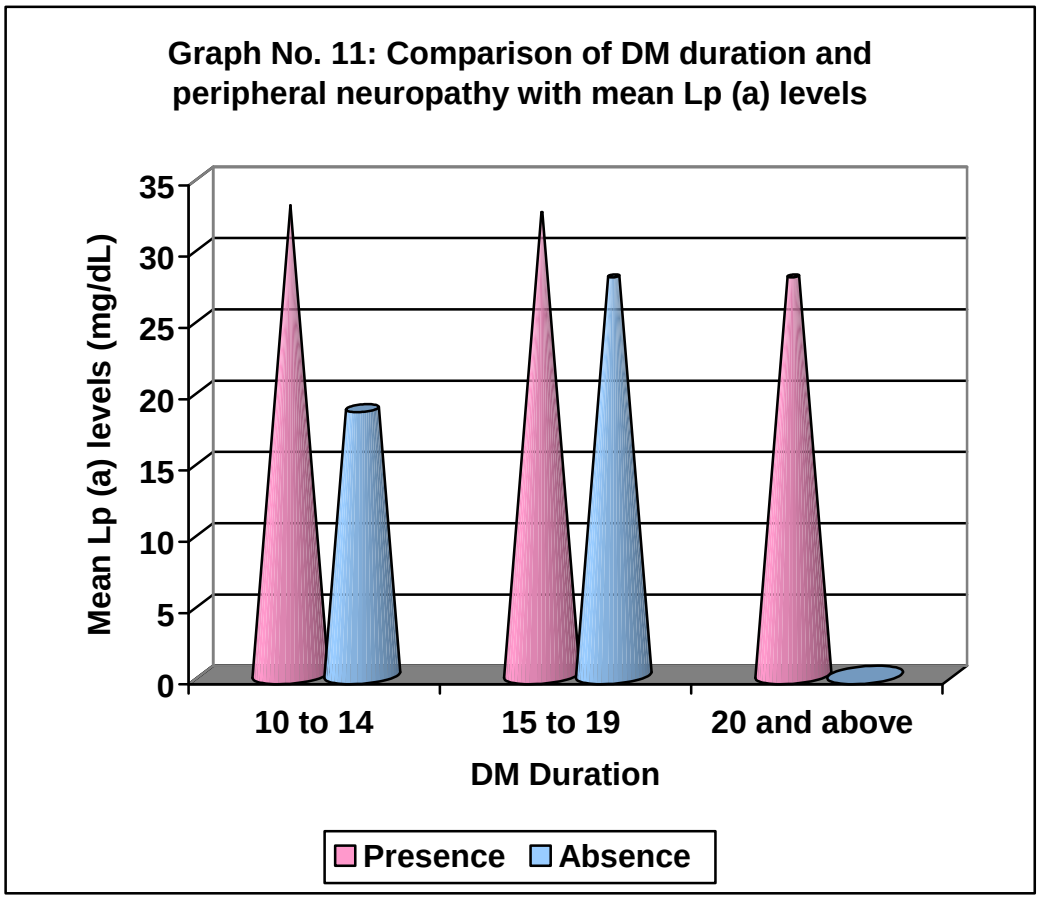


Among 50 diabetic patients 41 (82.0%) had peripheral neuropathy with mean Lp (a) levels of 19.76 mg/dL. Nine patients (18.0%) had no peripheral neuropathy with mean Lp (a) levels of 32.74 mg/dL. These findings were statistically significant (p=0.016) and also showed that among 50 diabetics majority had peripheral neuropathy in more than 10 years duration of diabetes.

Table No. 11: Comparison of DM duration and peripheral neuropathy with mean Lp (a) levels

DM duration (Years)	Peripheral Neuropathy	Number of patients		Mean Lp (a) levels (mg/dL)	
		Number	**Percentage**	**Mean**	**S.D.**
10 to 14	Present	32	64.0%	32.96	15.64
	Absent	08	16.0%	18.72	12.29
	Total	40	80.0%	30.11	15.96
15 to 19	Present	08	16.0%	32.46	10.79
	Absent	01	2.0%	28.00	-
	Total	09	18.0%	31.96	10.20
20 and above	Present	01	2.0%	28.00	-
	Absent	00	0.0%	-	-
	Total	01	2.0%	28.00	-
Total	Present	41	82.0%	32.74	14.51
	Absent	09	18.0%	19.75	11.91
	Total	**50**	**100.0%**	**30.40**	**14.85**

t=2.5 **p = 0.016**

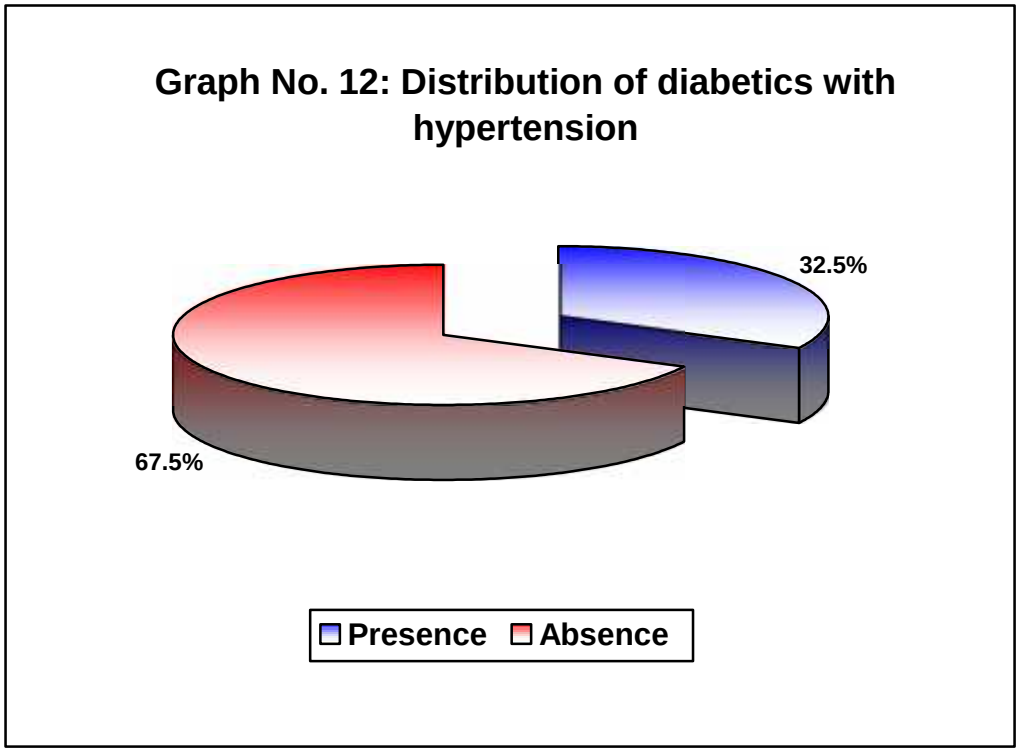


Those patients who had peripheral neuropathy had significantly higher Lp (a) levels compared to those who did not have peripheral neuropathy (p=0.016). There was also a comparative increase in the mean Lp (a) levels in patients of 10 to 14 years to 15 to 19 years age group and these findings were statistically significant (p = 0.016).

Table No. 12: Comparison of hypertension and mean Lp (a) in diabetics

Hypertension	Number of patients		Mean Lp (a) levels (mg/dL)	
	Number	**Percentage**	**Mean**	**S.D.**
Present	17	34.0.%	32.80	10.16
Absent	33	66.0%	25.76	16.39
Total	**50**	**100.0%**	**30.40**	**14.85**

t = 1.612 p = 0.114

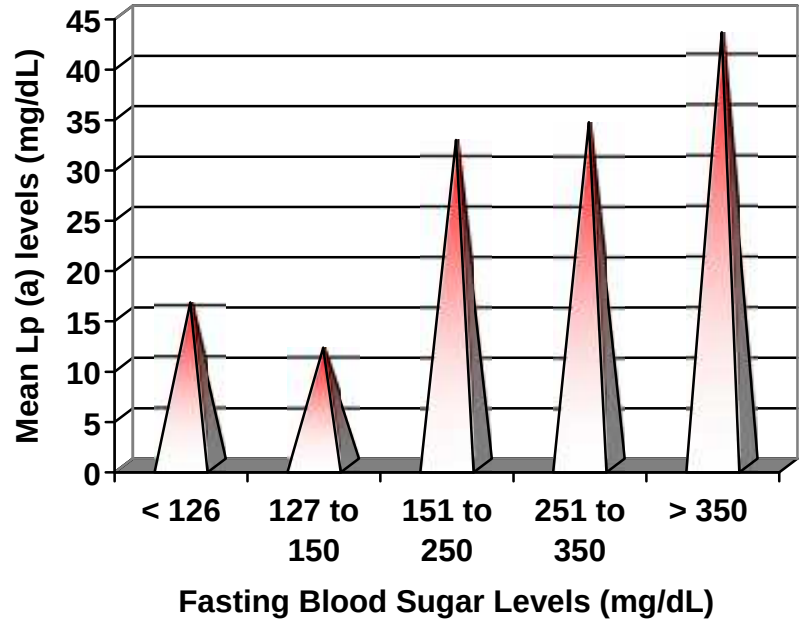


In the present study of 50 patients 67.5% of patients had hypertension with mean Lp (a) levels of 32.80 mg/dL whereas 32.5% did not had hypertension with mean Lp (a) levels of of 25.76 mg/dL. Though not statistically significant (p = 0.114), mean Lp (a) levels were found to be higher in patients with hypertension than compared to patient without hypertension.

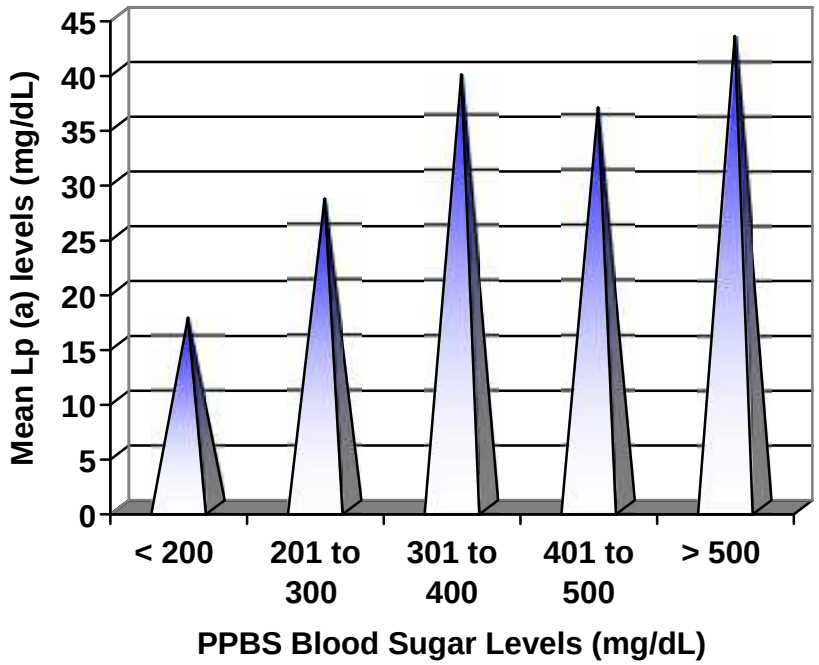
Table No. 13: Comparison of blood sugar levels and mean Lp (a)

Blood sugar levels (mg/dL)		Number of patients		Mean Lp (a) levels (mg/dL)	
		Number	**Percentage**	**Mean**	**S.D.**
FBS	Less than 126	05	10.0%	16.18	6.34
	127 to 150	02	4.0%	11.70	6.92
	151 to 250	33	66.0%	32.32	14.38
	251 to 350	09	18.0%	34.07	15.26
	More than 350	01	2.0%	43.00	-
	Total	**50**	**100.0%**	**30.41**	**14.85**
	x^2 = 70.00		f = 2.732		**p = 0.041**
PPBS	Less than 200	03	6.0%	17.30	8.69
	201 to 300	34	68.0%	28.16	12.86
	301 to 400	10	20.0%	39.51	18.49
	401 to 500	02	4.0%	36.50	19.00
	More than 500	01	2.0%	43.0	-
	Total	**50**	**100.0%**	**30.41**	**14.85**
	x^2 = 77.00		f = 2.173		p = 0.087

Graph No. 13: Comparison of fasting blood sugar levels with mean Lp (a) levels



Graph No. 14: Comparison of post prandial blood sugar levels with mean Lp (a) levels



The present study results suggested that as the FBS values increased, the mean Lp (a) levels also increased. Maximum mean Lp (a) levels were found in the group of FBS more than 350 mg/dL and F value revealed a significant difference between mean Lp (a) values of patients at different FBS levels.

While comparing PPBS levels with Lp (a) levels, the results suggested that as PPBS values increased, the mean Lp (a) levels also increased, except at PPBS level of 301 to 400 mg/dL. This could be due to well controlled diabetic status in these patients with insulin therapy. Maximum Lp (a) levels were found in the PPBS more than 550 mg/dL group. The F value revealed a non-significant difference between mean Lp (a) values of patients with different PPBS levels.

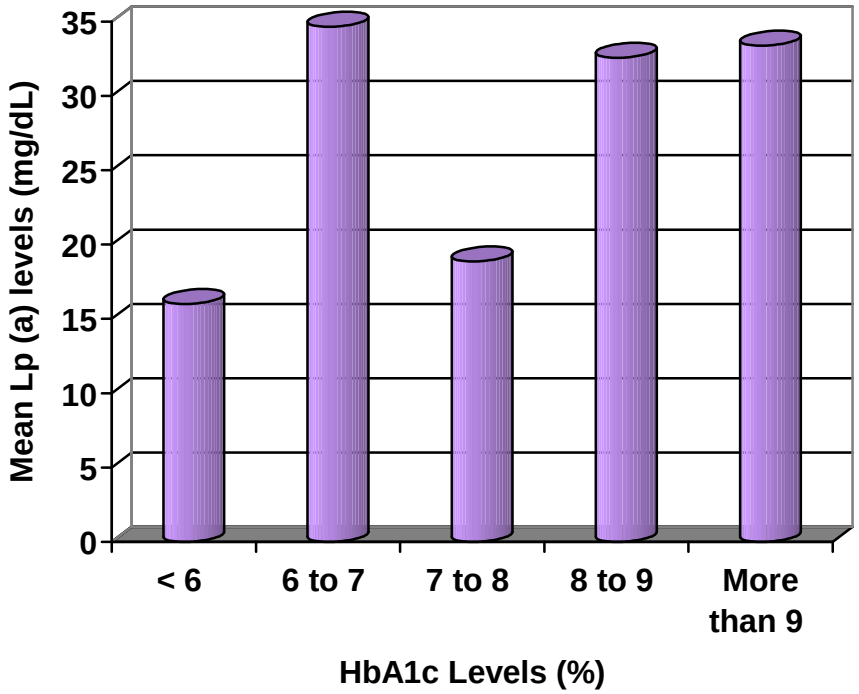
On the whole, the above two results indicate that better the glycemic control, lesser the mean Lp (a) values.

Table No. 14: Comparison of HbA1c and mean Lp (a)

HbA1c levels (%)	Number of patients		Mean Lp (a) levels (mg/dL)	
	Number	**Percentage**	**Mean**	**S.D.**
< 6	01	2.0%	16.00	-
6 to 7	04	8.0%	34.65	30.57
7 to 8	09	18.0%	18.86	9.29
8 to 9	07	14.0%	32.57	6.39
More than 9	29	58.0%	33.38	13.75
Total	**50**	**100.0%**	**30.41**	**14.85**

x^2 = 48.80 f = 2.203 p = 0.084

Graph No. 15: Comparison of HbA1c levels with mean Lp (a) levels



In the present study, 29 patients (58.0%) had HbA1c levels more than 9.0% with mean Lp (a) of 33.38 mg/dL. In seven patients had HbA1c levels between 8 to 9% with mean Lp (a) of 32.57 mg/dL, nine patients had HbA1c levels between 7 to 9% with mean Lp (a) of 18.86 mg/dL, four patients had HbA1c levels between 6 to 7% with mean Lp (a) of 34.65 mg/dL and one patient had HbA1c levels less than 6 with mean Lp (a) of 32.57 mg/dL. Though not statistically significant, the clear trend was observed, where the HbA1c levels increased, mean Lp (a) also increased linearly except for one HbA1c level. This probably could be because of well controlled diabetic status in these patients with insulin therapy.

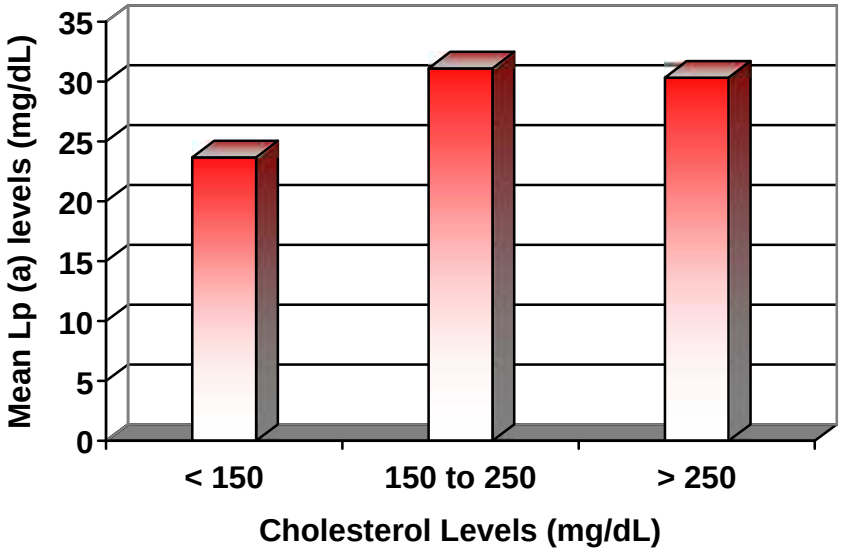
Comparison of mean Lp (a) levels with other lipids

Table No. 15: Comparison of serum cholesterol with mean Lp (a)

Cholesterol (mg/dL)	Number of patients		Mean Lp (a) levels (mg/dL)	
	Number	Percentage	Mean	S.D.
Less than 150	03	6.0%	23.67	4.93
150 to 250	31	62.0%	31.10	15.00
More than 250	16	32.0%	30.33	16.02
Total	**50**	**100.0%**	**30.41**	**14.85**

x^2 = 23.56 f = 0.333 p = 0.718

Graph No. 16: Comparison of cholesterol levels with mean Lp (a) levels



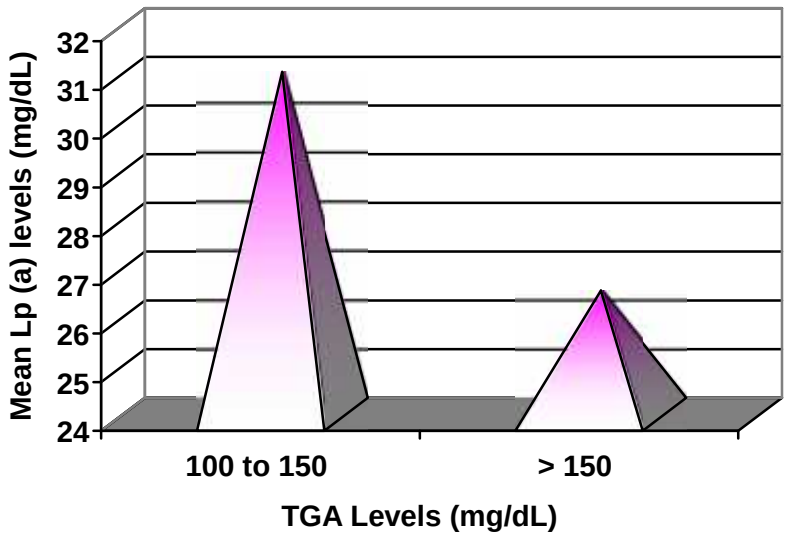
The present study showed that, 31 patients (62.0%) had cholesterol levels ranging from 150 to 250 mg/dL with mean Lp (a) of 31.10 mg/dL. Sixteen patients (32.5%) had cholesterol levels more than 250 mg/dL with mean Lp (a) of 30.33 mg/dL and three patients had cholesterol levels less than 150 with mean Lp (a) of 23.67 mg/dL. Eventhough no specific trend was observed with serum cholesterol levels and Lp (a) values ($p = 0.718$), mean Lp (a) levels were increased in patients with serum cholesterol level of 150 to 250 mg/dL.

Table No. 16: Comparison of TGA and Lp (a)

TGA levels (mg/dL)	Number of patients		Mean Lp (a) levels (mg/dL)	
	Number	**Percentage**	**Mean**	**S.D**
100 to 150	07	14.0%	26.54	11.82
More than 150	43	86.0%	31.03	15.30
Total	**50**	**100.0%**	**30.41**	**14.85**

t = -0.739 df = 48 p = 0.464

Graph No. 17: Comparison of TGA levels with mean Lp (a) levels

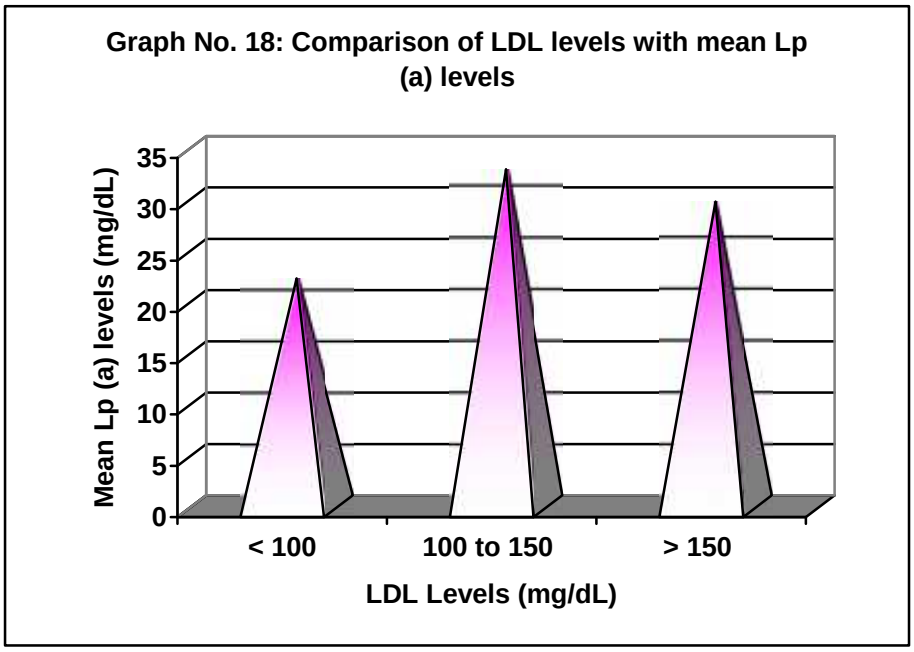


In the present study seven patients (14.0%) had serum TGA levels between 100 to 150 mg/dL with a mean Lp (a) levels of 26.54 mg/dL and 43 patients (86%) had TGA levels more than 150 mg/dL with a mean Lp (a) levels of 31.03 mg/dL. Even though no statistical significance was found, patients with TGA level of more than 150 mg/dL had increased levels of mean Lp (a) of 31.03 mg/dL.

Table No. 17: Comparison of serum LDL with mean Lp (a)

LDL (mg/dL)	Number of patients		Mean Lp (a) levels (mg/dL)	
	Number	**Percentage**	**Mean**	**S.D.**
Less than 100	06	12.0%	22.17	15.81
100 to 150	26	52.0%	32.81	17.29
More than 150	18	36.0%	29.68	9.42
Total	**50**	**100.0%**	**30.41**	14.85

x^2 = 6.200 f = 1.302 p = 0.282



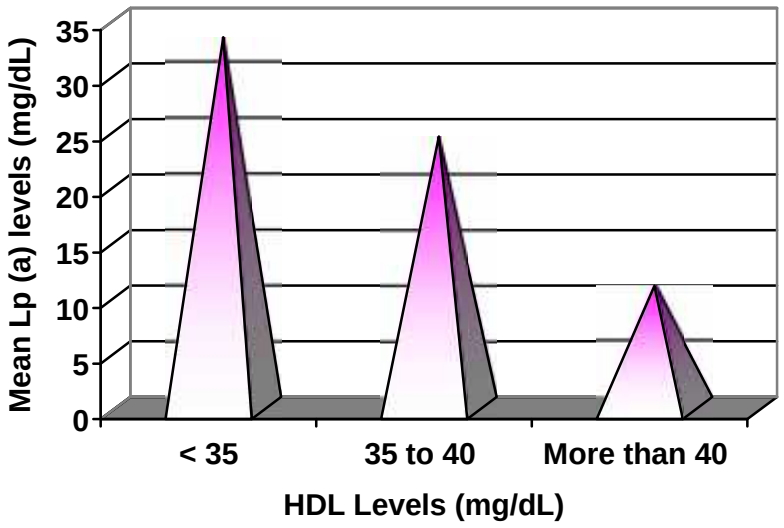
In the present study, 26 patients (52.0%) had LDL levels ranging from 100 to 150 mg/dL with mean Lp (a) of 32.81 mg/dL followed by 18 patients (36.0%) with LDL levels more than 150 with mean Lp (a) of 29.68 mg/dL and six patients (12.0%) had LDL levels less than 100 mg/dL with mean Lp (a) of 22.17 mg/dL. No specific trend was observed with serum cholesterol levels and mean Lp (a) values (p = 0.282) but mean Lp (a) values were increased in patients with LDL levels ranging from 100 to 150 mg/dL.

Table No. 18: Comparison of HDL and Lp (a)

HDL (mg/dL)	Number of patients		Mean Lp (a) levels (mg/dL)	
	Number	**Percentage**	**Mean**	**S.D.**
Less than 35	38	76.0%	33.35	14.92
35 to 40	09	18.0%	24.42	9.56
More than 40	03	6.0%	11.00	4.65
Total	**50**	**100.0%**	**30.41**	**14.85**

df = 2 f = 4.643 **p = 0.014**

Graph No. 19: Comparison of HDL levels with mean Lp (a) levels



Among 50 diabetic patients 38 (76.0%) patients had mean Lp (a) of 33.35 mg/dL followed by nine patients (18.0%) with HDL levels between 35 to 40 mg/dL had mean Lp (a) levels of 24.42 mg/dL and three patients (six percent) had HDL levels more than 40 mg/dL with mean Lp (a) level of 11 mg/dL. There was an inverse relationship between HDL and mean Lp (a) levels and was statistically significant (p = 0.014).

Table No. 19: Comparison of waist hip ratio and mean Lp (a)

WHR		Number of patients		Mean Lp (a) levels (mg/dL)	
		Number	**Percentage**	**Mean**	**S.D.**
Females	< 0.8	01	4.8%	6.80	0.00
	> 0.8	20	95.2%	26.30	11.80
	t = 1.612			p = 0.123	
Males	< 0.95	09	31.0%	35.33	9.12
	> 0.95	20	69.0%	33.48	18.05
	t = 0.291			p = 0.774	

In the present study, among 21 females 20 had WHR more than 0.8 and in them the mean Lp (a) levels (26.30 mg/dL) were increased. No similar trend was found in males. Among 29 males 20 had WHR more than 0.95 with mean Lp (a) levels of 33.48 mg/dL.

Table No. 20: Comparison of body mass index and Lp (a)

BMI	Number of patients		Mean Lp (a) levels (mg/dL)	
	Number	**Percentage**	**Mean**	**S.D.**
20 to 25	18	36.0%	29.03	12.40
25.1 to 27	20	40.0%	32.61	16.00
27.1 to 30	10	20.0%	29.93	18.34
More than 30	02	4.0%	23.00	7.07
Total	**50**	**100.0%**	**30.40**	**14.85**

x^2 = 81.066 df = 3 p = 0.787

Even though no statistical significance was found between BMI and mean Lp (a) levels the results suggested that mean Lp (a) levels were maximum (32.61 mg/dL) in the obese group.

DISCUSSION

Diabetes Mellitus is a chronic disease that requires long term medical attention both to limit the development of its devastating complications and to manage them when they do occur. Therefore the present study has been undertaken to assess the serum concentration of Lp (a) levels and different microvascular and macrovascular complications which develop in type 2 DM patients. In the present study, 50 patients were included who were detected to have laboratory proven type 2 DM for more than 10 years. These diabetic patients were either on diet with oral hypoglycaemic agents (OHA) and or on insulin therapy. The metabolic status, prevalence of diabetes and various diabetic complications were assessed by detailed history, physical examination, limited vascular and neurological examination and laboratory and ECG analysis.

Mean Lipoprotein (a) and age and sex

In the present correlation study of 50 diabetic patients, the serum levels of Lp (a) was studied in patients, aged between 40 to 70 years with male : female ratio of 1.3:1 with duration of diabetes varying from 10 years to 20 years. The maximum number of patients belonged in the 61 to 70 years of age group. A highly significant relationship was found between Lp (a) and increasing age in these patients.

In this study, we found that males had higher serum Lp (a) levels (34.05 mg/dL) than female group (25.37mg/dL). This could be due to increase number of males of 29 (58%) among 50 study population compared to 21 (42%) females.

Hence it may be concluded that Lp (a) levels are affected by sex and age. Another study on Lp (a) levels in the Japanese population with reference to influence of age and sex ,the authors found that Lp (a) increased with age and the values were statistically significant and were higher in females than in males.[85] In the Framingham offspring study, the mean Lp (a) levels in both genders were measured and showed similar results.[86] The findings in these study substantiated the present study.

Mean Lipoprotein (a) and race

The present study was done in Asian Indians who were diabetic for more than 10 years. The results of the study revealed that, the mean Lp (a) levels were increased in these population. And there was a strong correlation between mean Lp (a) levels and microvascular complications of diabetes in them. Higher levels of Lp (a) compared to other ethnic groups have been recorded in Asian Indians in US, UK, and Singapore.[87,88,89] Further evidence has been provided by another study where mean levels of Lp (a) were nearly double in sons of Asian Indians with CAD compared to similar-aged sons of white parents.[90]

Tibetans, Japanese and Koreans have Lp (a) levels similar to the Caucasians. Higher Lp (a) levels are observed in blacks.[91] Close correlation between CAD and Lp (a) levels has been observed in Welsh, Germans, Swedish, Finnish, Icelanders, Austrians, Australians, Chinese, and Japanese.[92,93]

Microvascular complications in type 2 DM and their correlation with serum Lipoprotein (a) levels

Lipoprotein (a)and retinopathy

In the present study of 50 patients, ocular fundi were examined by both direct and indirect ophthalmoscopy after mydriasis.

The findings were graded as:

- No signs of diabetic retinopathy
- Non proliferative diabetic retinopathy (NPDR)
- Proliferative diabetic retinopathy.

Individuals were classified as having PDR, if they had new vessels, vitreous haemorrhage, vitreoretinal traction or retinal detachment believed to be attributable to diabetic neovascularisation. NPDR and PDR were taken together as retinopathy for this study.

The relationship of the serum Lp (a) concentration with the presence and absence of diabetic retinopathy were statistically evaluated. Among 50 diabetic patient, even though only 17 (34%) had retinopathy, the mean Lp (a) levels (31.87 mg/dL)were found to be increased than compared to mean Lp (a) (17.22 mg/dL) in 33 (66%) patients without diabetic retinopathy(p 0.035). And when compared with duration of type 2 DM in these patients, even though statistically insignificant, none of the patients were devoid of retinopathy when the DM duration was more than 15 years.

In a study of Lp (a) levels in type 2 DM patients with retinopathy, it was found that the proliferative diabetic retinopathy after 10 years of diabetes were found to be 6.5 fold higher , and in them the serum Lp (a) levels were found to be high ($p < 0.05$).[94]

Another study of high serum Lp (a) levels in 412 Korean type 2 DM patients with proliferative diabetic retinopathy concluded that diabetic patients with proliferative diabetic retinopathy had higher serum Lp (a) levels versus those with no diabetic retinopathy or with non proliferative diabetic retinopathy.[95]

A study on Lp (a) as a risk factor for retinopathy in patient with type 2 DM, among 200 patients of 100 cases with retinopathy and 100 controls, average Lp (a) levels in the study group was 68.5 mg/dL and was significantly higher than in control group with Lp (a) level of 25.1 mg/dL ($p = 0.001$).[96] The Lp (a) levels were found to be increasing with increasing severity of diabetic retinopathy. They concluded that serum Lp (a) levels are significantly raised in patients with diabetic retinopathy as compared to those with no retinopathy. Also a study in 194 patients of NIDDM, they found that higher Lp (a) levels are associated with higher prevalence of retinopathy.[80] These results suggests that Lp (a) might play a role in the occlusion of retinal capillaries leading to PDR. But further prospective studies are required to prove the causal relationship.

Lipoprotein (a) and diabetic nephropathy

In the present study, 27 patients had serum creatinine less than one mg/dl with mean Lp (a) of 29.96 mg/dL and nine patients had creatinine levels between 1 to 1.5 mg/dL with mean Lp (a) levels of 28.74 mg/dL and four patients had

creatinine levels of more than 1.5 mg/dL with mean Lp (a) level of 35.61 mg/dL .When serum Lp (a) levels were compared with serum creatinine levels, the mean Lp (a) levels were found to be progressively increasing ,but it was statistically insignificant. But previous studies revealed that patients with nephropathy had significantly higher Lp (a) levels than patients without nephropathy. .Hence it made us further provoke into the issue and we calculated creatinine clearance in these study population and it showed that as creatinine clearance decreased (ECCR of 21 to 50 had mean Lp (a) of 38.25 mg/dl and ECCR of more than 80 had mean Lp (a) of 30.40 mg/dL (p 0.040). A significant inverse correlation of serum Lp (a) with effective creatinine clearance and a statistically significant correlation between the effective creatinine clearance and mean Lp (a) levels were observed. Also very high levels Lp (a) levels have been documented in patients with non diabetic chronic renal failure.[97]

This suggests that renal dysfunction is associated with raised serum Lp (a) levels. Patients with nephropathy, proteinuria (microalbuminuria), with high levels of Lp (a) are probably because of decreased renal excretion, rather than raised Lp (a) causing nephropathy. It can be thus concluded that serum Lp (a) levels are raised in diabetic nephropathy and probably it is due to renal dysfunction.

Lipoprotein (a) and neuropathy

In the present study of 50 diabetic patients, Diabetic neuropathy was scored by the criteria of Boulton:[98]

0 - none

1 - Complaints suggestive of peripheral neuropathy

2 - decreased vibration sense or signs of sensory disturbance without complaints

3 - Sensory complaints and reduced vibration sense or sensory defects.

Out of 50 patients 82% had peripheral neuropathy with mean Lp (a) levels of 32.74 mg/dL and 18% had no peripheral neuropathy with mean Lp (a) levels of 19.76mg/ dL. These findings were statistically significant (p= 0.016) and when compared with duration of diabetes it also showed that among 50 diabetics majority had neuropathy in more than 10 years duration of diabetes.

In a study on Lp (a) levels in type 2 DM subjects in relation to diabetic microvascular complications on 200 patients the mean Lp (a) in patients with neuropathy and without neuropathy were similar (28.9 mg/dL) vs 29.3 mg/ dL (p>0.92).[99] But the present study had a positive finding. In a 25 years prospective study in 4,400 unselected diabetics, 12% of patients had signs and symptoms of diabetic neuropathy at the time of diagnosis. Thereafter a linear increase and 50% of patients developed diabetic neuropathy in 25 years.[100]

In another study of the longitudinally examined type 2 diabetics patients 54% had neuropathy with increased levels of Lp (a).[101]

Serum Lipoprotein (a) levels and lipid profile in diabetic patients

Our study conducted in 50 patients with diabetes mellitus revealed statistically significantly decreased concentrations of HDL cholesterol with increased mean Lp (a) levels (HDL less than 35 mg/ dL had mean Lp (a) of 33.35

mg/dL) suggesting an inverse correlation between HDL and mean Lp (a) levels. While comparing TGA and Lp (a), study revealed increased concentration of TGA had increased levels of mean Lp (a) levels. (TGA>150 mg/dL, mean Lp (a) of 31.03 mg/dL). However they were also statistically insignificant. Even though not statistically significant the mean Lp (a) levels (31.10 mg/dL) were found to be increased in patients with moderate increase in serum cholesterol levels of 150 to 250 mg/dL. The increased mean Lp (a) of 32.81 mg/dL were also found in diabetic patients with serum LDL levels of 100 to 150 mg/dL. However they were statistically insignificant. These results of the study reveals that probably the patients with type 2 DM develop metabolic changes that impair lipoprotein metabolism leading to hyperlipidemia and accelerates the development of atheromatous and vascular complications.

In a study on serum Lp (a) and lipid profile in young South Indian patients with MI had results suggesting that high level of Lp (a) and TG/HDL ratio has a distinctive association with MI, independent of other common coronary risk factors.[102] Studies performed in the native Indian population also record increased levels of Lp (a) in patients with atherosclerotic vascular diseases.[103] In another study, Lp (a) levels in 114 consecutive patients undergoing coronary angiography were compared with controls. CAD patients had higher levels of Lp (a).[104] In another study done on North Indian patients, apo (a) phenotypic polymorphism and its effect on Lp (a) levels was studied on 130 angiographically proven CAD patients and 130 age and sex matched controls.[105]

Quartile distribution of serum Lp (a) levels (mg/dL) in CAD patients and controls

Group	Mean	S.D.	1 – 25	50	75 – 100	Skew
Patients (n=130)	42	24	0.99 -16.0	31.1	62.9-160	1.21
Controls (n=130	27	27	0.69-7.8	17.7	36.6-147	2.13

Additionally, a more recent study has demonstrated that in men with coronary artery disease and elevated LDL levels, Lp (a) was a major factor in determining disease severity, progression, and event rate.[106]

In a meta-analysis, 12 out of the 14 prospective studies showed Lp (a) concentration to be increased in subjects later developing CAD.[107]

Levels of HDL are generally decreased in men and women with diabetes. Another study, in which investigators measured differences in HDL levels between men (n=1846) and women (n=2703) with and without diabetes. The researchers reported that men and women with diabetes had lower levels of HDL than men and women without the disease, and that this difference was greatest in women.[108] These data may explain why women with diabetes have an increased relative risk for cardiovascular disease compared with men.

An 11 year prospective study of men 43 to 54 years of age indicated that hypertriglyceridemia among subjects with impaired glucose tolerance or diabetes was significantly associated with coronary death.[109] A study investigated the relationship between lipoprotein fractions and the risk of CHD in patients with type 2 diabetes. Patients were followed for seven years, and all CHD events (CHD death or CHD events, including nonfatal MI) were recorded. Of the

original 313 diabetic patients (153 men and 160 women) studied, 56 patients (28 men and 28 women) died from CHD and 25 (17 men and 8 women) had a nonfatal MI during the follow-up period.[110]

In the above comparison study of HDL and VLDL triglycerides, HDL and VLDL triglycerides were both found to be inversely associated with CHD events in type 2 diabetes patients with low HDL levels (more than or equal to 43 mg/dL). The investigators concluded that their 7-year follow-up study provided evidence that low HDL and HDL2 cholesterol, high VLDL cholesterol, and high total and VLDL triglycerides are powerful risk indicators for CHD events in patients with type 2 diabetes.

Prospective studies have been performed to more directly assess the contribution of Lp (a) to the development of coronary artery disease. In these studies, fewer in number than the case-control studies, the results have been some what discordant.[111] Whereas six of the prospective studies concluded that Lp (a) concentration is a risk factor for myocardial infarction or coronary artery disease in men, three other nested case-control studies did not reach the same conclusion.[112] Possible explanations for the conflicting results may include differences in experimental design, for example sample size, stringency of exclusion criteria, length of the follow-up period, collection and storage condition of samples, choice of analytical method for Lp (a) measurement, and the statistical approaches utilized for data analysis. Discrepancies in outcomes may also reflect population differences, the impact of which is poorly understood at present.

Another study also reported a higher prevalence of low-molecular weight apo (a) isoforms in female (but not male) myocardial infarction survivors, compared with controls.[113] Similarly, two additional studies reported a higher prevalence of small Apo (a) isoforms in familial hypercholesterolemia patients with coronary artery disease than in those without coronary artery disease.[114] In the Stanford Five-City Project prospective case-control study, Wild and coworkers reported that Lp (a) is an independent risk factor for the development of coronary artery disease in men and that the size of apo (a) may also play a role in this process.[115] In this study, neither Lp (a) levels nor apo (a) isoform sizes were found to be significantly different between female cases and control subjects. It should be noted, however, that the study contained a relatively small number of case-control pairs (90 male and 44 female). Clearly, additional studies employing larger sample sizes are required to better define the contribution of Lp (a) size heterogeneity to risk of disease in men and women and in different ethnic groups.

The Framingham Heart Study provided data from a large prospective study assessing the effect of elevated Lp (a) levels on risk for cardiovascular disease in women. In this study, the presence of a sinking pre-3-lipoprotein band in electrophoresis, indicating elevated plasma Lp (a), was found to be a strong, independent predictor of myocardial infarction, intermittent claudication, and cardiovascular disease in 3,003 women observed for a mean of 12 years. However, Lp (a) values were not directly measured in this study, and the apo (a) isoforms were not determined.[116] Therefore, the association between Lp (a) levels and coronary artery disease and the contribution of apo (a) size to Lp (a)

atherogenicity in women still need to be addressed.

In a prospective study of 10 years followup of Lp (a) and risk of CHD among women with type 2 DM they found the probability of developing CHD over 10 years was higher among diabetic women with substantially higher levels of both LP (a) (more than 1.07 mmol/L) and triglycerides (more than 2.5 mmol/L) than among diabetic women with lower levels.[117]

Diabetic women with a higher level of only Lp (a) or TGs had a similar 14% risk.

In another study, Lp (a) and comprehensive lipid tetrad index as a marker for CHD in NIDDM patients in South India showed Lp (a) concentration had showed strong correlation with CAD in NIDDM patients of South India.[118] High concentration of LP (a) and comprehensive lipid tetrad index along with high prevalence of NIDDM, may render Indians particularly vulnerable to malignant atherosclerosis at young age.[87] Hence Lp (a) level in serum emerges to be a promising marker for the diagnosis of coronary artery disease.

Lp (a) and hypertension

Our study demonstrated a significant correlation of serum Lp (a) with hypertension in diabetic patients, suggesting that Lp (a) levels in diabetic patients may have important implications for the increased susceptibility to vascular disease in these patients. A study on association of serum Lp (a) with hypertension in diabetic patients suggests that Lp (a) is an independent determinant of kidney function in diabetic patients with hypertension.[119]

Recent studies suggest that Lp (a) can act as a marker for determining vascular or tissue injury.[120,121] In a study conducted on a total of 37 diabetic patients with essential hypertension, it was observed that the hypertensive patients had higher plasma concentrations of Lp (a) (more than 30 mg/dl), total cholesterol (TC), low-Density lipoprotein-cholesterol, and triglycerides than controls. Lp (a) values correlated significantly with systolic blood and diastolic blood pressures.[119] In a similar study, it was reported that there was significantly elevated levels of plasma Lp (a) in 123 Caucasian essential arterial hypertensive patients.[119] Recent report suggests that arterial hypertension is associated with elevated Lp (a) levels in patients of end-stage renal disease. In their study, it was observed that Lp (a) levels were significantly higher in the hypertensive patients, but that difference was not significant from the non-renal failure patients.

In conclusion, our results suggest that serum Lp (a) concentration aggravate hypertension and identify kidney failure as a primary determinant of raised Lp (a) in diabetic patients. Association of Serum Lipoprotein (a) with hypertension in diabetic patients has important implications for the increased susceptibility to vascular disease associated with Lp (a) in diabetic patients.

Lp (a) and blood sugar levels

In the present study, we found that as FBS values increase, the mean Lp (a) levels also increased. Maximum Lp (a) levels were found in the FBS more than 350 mg/dL group and it was statistically significant. Also we found that as PPBS values increased, the mean Lp (a) levels also increased except at PPBS level of 301 to 400 mg/dl this could be because of well controlled diabetic status

in these patients with insulin therapy. The correlation between mean Lp (a) and PPBS were also statistically significant (p=0.08). Similar findings were observed when mean Lp (a) levels were compared with HbA1c. On the whole the results of this study indicate that better the glycemic control, lesser the mean Lp (a) levels.

In a study on Lipoprotein(a) concentration in non-insulin-dependent diabetes mellitus and borderline hyperglycemia there was no correlation between Lp (a) levels and FPG (p=.030)and they concluded that Lp (a) levels are similar in individuals with NIDDM, borderline hyperglycemia, and normoglycemia.[122] Another study on Lp (a) revealed that mean Lp (a) levels were not influenced by type of hypoglycemic treatment or blood glucose level, and Lp (a) concentrations are not related to glycemia.[123] In another study on Insulin and 2-Hour Glucose Levels Are Inversely Related to Lp (a) Concentrations Controlled for LPA Genotype. There were significant correlations of Lp (a) with insulin and glucose concentrations, especially for insulin.[124] Hence it may be concluded that Lp (a) is another, among many lipoproteins, that is associated with the metabolic syndrome characterized by insulin resistance.

Lp (a) and WHR and BMI

Type of diabetes and waist-hip ratio are important determinants of serum lipoprotein (a) levels in diabetic patients. In our analysis, we included waist hip ratio because it was the most common obesity-related predictor of diabetes after body mass index and it has a weaker correlation with body mass index ($r \approx 0.4$)[125] and the present study could not demonstrated consistently strong associations of body mass index, and WHR with mean Lp (a) levels in type 2 DM patients as

compared to a study on metabolic and anthropometric determinants of serum Lp (a) concentrations and Apo(a) polymorphism in Arab population.[126] Though found to be non significant, in the present study female patients had increased mean Lp (a) levels as the WHR increased. No specific trend was observed when mean Lp (a) levels were compared with BMI. Probably further prospective studies can demonstrate a strong associations of body mass index, and waist/hip ratio with mean Lp (a).

In conclusion the serum concentration of Lp (a) levels are elevated in different microvascular and macrovascular complications which develop in type 2 DM patients.

Although, undoubtedly of importance in diabetic dyslipidemia high Lp (a) levels is likely to be but one of the many reasons for the accelerated macrovascular and microvascular disease in diabetic patients. The treatment of lipid abnormalities has the potential to reduce these complications more than 50%. A clinician with an owl's eye can effectively use Lp (a) as a predictive tool to evaluate the complications of type 2 DM in terms of its onset and progression. This will help a diabetic patient to have array of hope to lead a healthy and a longer life.

CONCLUSION

The present correlation study was conducted in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum on 50 patients, detected to have laboratory proven type 2 DM for more than 10 years duration. The serum Lp (a) levels and the association with micro and macro vascular complications of DM were studied for a period of one year during January 2007 to December 2007. After analyzing the results the following conclusions were drawn.

- The Lp (a) values progressively increased as age increased.
- Lp (a) levels were maximum between 61 to 70 years.
- Patients with retinopathy, nephropathy and neuropathy had statistically significant increased levels of Lp (a). But the effective creatinine clearance ratio in these diabetics had an inverse relation to mean Lp (a) levels.
- Present study indicated that better the glycemic control, lesser the mean Lp (a) levels.
- Lp (a) levels were increased in patients with increased cholesterol and TGA levels (100 to 150 mg/dL). But Lp (a) was inversely proportionate to HDL levels. Hence Lp (a) levels may be used as a screening or predictive tool for development of coronary artery disease.

Hence Lp (a) may be an independent risk factor for the development of different complications of type 2 DM. However further studies on larger sample size may be helpful to confirm the above findings.

SUMMARY

Diabetes is the most common endocrine disorder and a burdensome chronic disease in the world today. Dyslipidemias frequently associated with type 2 DM are hypertriglyceridemia, hypercholesterolemia, postprandial lipemia, low HDL, preponderance of small and dense LDL, and elevated levels of Lp (a).

The objective of the present study was to assess the vascular complications of type 2 DM with respect to their serum levels of Lp (a).

The present correlation study was conducted in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum on 50 patients, detected to have laboratory proven type 2 DM for more than 10 years duration. The serum Lp (a) levels and the association with micro and macro vascular complications of DM were studied for a period of one year during January 2007 to December 2007.

The results of the study showed that, Lp (a) values progressively increased as age increased. DM patients with microvascular complications like retinopathy, nephropathy and peripheral neuropathy had increased levels of mean Lp (a) levels. Present study indicated that better the glycemic control, lesser the mean Lp (a) levels. As the sugar and other lipid levels increased Lp (a) levels were also increased except HDL wherein an inverse proportion was observed. Lp (a) levels may be used as a screening or predictive tool for development of coronary artery disease. Hence Lp (a) may be an independent risk factor for the development of different complications of type 2 DM.

BIBLIOGRAPHY

1. Mann JI, Lewis B, Shepherd J, Winder AF, Fenster S, Rose L et al. Blood lipid concentrations and other cardiovascular risk factors. Br Med J 1988; 296: 1702-6.

2. Marcovina SM, Koschinsky ML. Lipoprotein (a) as risk factor for coronary artery disease. Am J Cardiol 1998; 82; 57U-66U.

3. Deepa R, Mohan A, Rema M, Haranath SP, Saravanan G, Mohan V. Lipoprotein (a) in South Indian type II diabetic subjects in relation to diabetic vascular complications. J Assoc Physicians India 2002; 50(5): 657-61.

4. Ki-Ho Song, Seung Hyun Ko, Hyung-Wook Kim, Yu-Bae Ahn, Jong-Min Lee, Hyun-Shik Son et al. Prospective study of lipoprotein (a) as a risk factor for deteriorating renal function in type II diabetic patients with overt proteinuria. Diabetes Care 2005; 28(7): 1718-23.

5. Wakabayashi I, Masuda H. Lipoprotein (a) as a determinant of arterial stiffness in elderly patients with type II diabetes mellitus. Clinica Chemica Acta 2006; 373(1-2): 127-31.

6. Eaton DL, Fless GM, Kohr WJ, McLean JW, Xu QT, Miller CG, et al. Partial amino acid sequence of apolipoprotein(a) shows that it is homologous to plasminogen. Proc Natl Acad Sci U S A. 1987; 84(10): 3224-8.

7. Lindahl G, Gersdorf E, Menzel HJ, Duba C, Cleve H, Humphries S, et al. The gene for the Lp(a)-specific glycoprotein is closely linked to the gene for plasminogen on chromosome 6. Hum Genet. 1989; 81(2): 149-52.

8. Luthra K, Misra A, Srivastava LM. Lipoprotein (a): biology and role in atherosclerotic vascular disease. Curr Sci 1999; 76: 1553–60.

9. Miles LA, Fless GM, Levine EG, Scanu AM, Plow EF. A potential basis for the thrombotic risks associated with lipoprotein (a). Nature 1989; 339: 301-3.

10. Zipes DP, Libby P, Bonow RO, Braunwald E. Braunwald's Heart Disease – A textbook of cardiovascular medicine. 7th Ed. Philadelphia: Saunders; 2005.

11. Lobentanz EM, Dieplinger H. Biogenesis of lipoprotein (a) in human and animal hepatocytes Electrophoresis. 1997; 18(14): 2677-81.

12. Chiesa G, Hobbs HH, Koschinsky ML, Lawn RM, Maika SD, Hammer RE. Reconstitution of lipoprotein (a) by infusion of human low density lipoprotein into transgenic mice expressing human apolipoprotein (a). J Biol Chem 1992; 267(34): 24369-74.

13. Reblin T, Niemeier A, Meyer N, Willnow TE, Kronenberg F, Dieplinger H, et al. Cellular uptake of lipoprotein [a] by mouse embryonic fibroblasts via the LDL receptor and the LDL receptor-related protein. J Lipid Res 1997; 38(10): 2103-10.

14. Knight BL. Lp (a) catabolism in hypercholesterolaemic individuals. Chem Phys Lipids 1994; 67-68: 233-9.

15. Huby T, Chapman J, Thillet J. Pathophysiological implication of the structural domains of lipoprotein (a). Atherosclerosis 1997; 133(1): 1-6.

16. Kostner KM, Clodi M, Bodlaj G, Watschinger B, Horl W, Derfler K, et al. Decreased urinary apolipoprotein (a) excretion in patients with impaired renal function. Eur J Clin Invest 1998; 28: 447-52.

17. Kostner GM, Wo X, Frank S, Kostner K, Zimmermann R, Steyrer E. Metabolism of Lp (a): assembly and excretion. Clin. Genet 1997; 52: 347-54.

18. Kostner KM, Kurt H, Stefenelli T, Rinner H, Maurer G. Urinary apo (a) discriminates coronary artery disease patients from controls. Atherosclerosis 1997; 129: 103-10.

19. Sandholzer C, Hallman DM, Saha N, Sigurdsson G, Lackner C, Császár A, et al. Effects of the apolipoprotein (a) size polymorphism on the lipoprotein (a) concentration in 7 ethnic groups. Hum Genet 1991; 86(6): 607-14.

20. Trommsdorff M, Kochl S, Lingenhel A, Kronenberg F, Delport R, Vermaak H, et al. A pentanucleotide repeat polymorphism in the 5 control region of the apolipo-protein(a) gene is associated with lipoprotein(a) plasma concentrations in Caucasians. J Clin Invest 1995; 96: 150-7.

21. Mooser V, Mancini FP, Bopp S, Petho-Schramm A, Guerra R, Boerwinkle E, et al. Sequence polymorphisms in the apo (a) gene associated with specific levels of Lp(a) in plasma. Hum Mol Genet 1995; 4: 173-81.

22. Tsai YH, Park S, Snook JT. Interaction among Lp (a) phenotypes, Lp (a) concentrations and lipoprotein response to fat-modified diets. J Nutr Biochem 1998; 9: 106-13.

23. Clevidence BA, Judd JT, Schaefer EJ, Jenner JL, Lichtenstein AH, Muesing RA, et al. Plasma lipoprotein (a) levels in men and women consuming diets enriched in saturated, cis-, or trans-monounsaturated fatty acids. Arterioscler Thromb Vasc Biol 1997; 17: 1657-61.

24. Bottalico LA, Keesler GA, Fless GM, Tabas I. Cholesterol loading of macrophages leads to marked enhancement of native lipoprotein (a) and apoprotein (a) internalization and degradation. J Biol Chem 1993; 268: 8569-73.

25. Schlaich MP, John S, Langenfeld MR, Lackner KJ, Schmitz G, Schmieder RE. Does lipoprotein(a) impair endothelial function? J Am Coll Cardiol 1998; 31: 359-65.

26. Allen S, Khan S, Tam S, Koschinsky M, Taylor P, Yacoub M. Expression of adhesion molecules by lp (a): a potential novel mechanism for its atherogenicity. FASEB J 1998; 12: 1765-76.

27. Liu AC, Lawn RM. Lipoprotein (a) and atherogenesis. Teratog Carcinog Mutagen 1994; 4: 40-4.

28. Etingen OR, Hajjar DP, Hajjar KA, Harpel PC, Nachman RL. Lipoprotein (a) regulates plasminogen activator inhibitor - 1 expression in endothelial cells. A potential mechanism in thrombogenesis. J Biol Chem 1991; 266: 2459-65.

29. Levin EG, Miles LA, Fless GM, Scanu AM, Baynham P, Curtiss LK, et al. Lipoproteins inhibit the secretion of tissue plasminogen activator from human endothelial cells. Arterioscler Thromb Vasc Biol 1994; 14: 438-42.

30. Karmansky I, Gruener N. Roles of immobilized glycosylated proteins and lipoprotein (a) in adhesivity of endothelial cells: possible implications for atherogenesis. Cell Biol Int 1992; 16: 503-15.

31. Poon M, Zhand X, Dunsky KG, Taubman MB, Harpel PC. Apolipoprotein (a) induces monocyte chemotactic activity in human vascular endothelial cells. Circulation 1997; 96: 2514-9.

32. Scanu AM, Fless GM. Lipoprotein (a). Heterogeneity and biological relevance. J Clin Invest 1990; 85: 1709-15.

33. Berg K, Dahlen G, Christophersen B, Cook T, Kjekshus J, Pedersen T. Lp (a) lipoprotein level predicts survival and major coronary events in the Scandinavian Simvastatin Survival Study. Clin Genet 1997; 52: 254-61.

34. Cantin B, Gagnon F, Moorjani S, Despres JP, Lamarche B, Lupien PJ, et al. Is lipoprotein(a) an independent risk factor for ischemic heart disease in men? The Quebec Cardiovascular Study. J Am Coll Cardiol 1998; 31: 519-25.

35. Djurovic S, Berg K. Epidemiology of Lp (a) lipoprotein: its role in atherosclerotic/thrombotic disease. Clin Genet 1997; 52(5): 281-92.

36. Grundy SM. Small LDL, atherogenic dyslipidemia and the metabolic syndrome (Editorial). Circulation 1997; 95: 1-4.

37. Napoli C, Triggiani M, Palumbo G, Condorelli M, Chiariello M, Ambrosio G. Glycosylation enhances oxygen radical-induced modifications and decreases acetylhydrolase activity of human low density lipoprotein. Basic Res Cardiol 1997; 92: 96-105.

38. Bucala R, Makita Z, Koschinski T, Cerami A. Lipid advanced glycosylated pathway for lipid oxidation in vivo. Proc Natl Acad Sci U S A 1993; 91: 9441-5.

39. Duell PB, Oram JF, Bierman EL. Nonenzymatic glycosylation of HDL and impaired HDL-receptormediated cholesterol efflux. Diabetes 1991; 40: 377-84.

40. Krauss RM. Heterogeneity of plasma low-density lipoproteins and atherosclerosis risk. Curr Opin Lipidol 1994; 5: 339-49.

41. Haffner SM, Mykkanen L, Festa A, Burke JP, Stern MP. Insulin-resistant prediabetic subjects have more atherogenic risk factors than insulin-

sensitive prediabetic subjects: implications for preventing coronary heart disease during the prediabetic state. Circulation 2000; 101: 975-80.

42. Ginsberg H, Plutzky J, Sobel BE. A review of metabolic and cardiovascular effects of oral antidiabetic agents: beyond glucose-level lowering. J Cardiovasc Risk. 1999; 6: 337-46.

43. Goldberg IJ. Clinical review 124: Diabetic dyslipidemia: Causes and consequences. JCE & M 2001; 86 (3): 965-71.

44. Dixon JL, Furukawa S, Ginsberg HN. Oleate stimulates secretion of apolipoprotein B-containing lipoproteins from Hep G2 cells by inhibiting early intracellular degradation of apolipoprotein B J Biol Chem 1991; 266: 5080-6.

45. Taghibiglou C, Carpentier A, Van Iderstine SC, Chen B, Rudy D, Aiton A, et al. Mechanisms of hepatic very low density lipoprotein overproduction in insulin resistance. Evidence for enhanced lipoprotein assembly, reduced intracellular ApoB degradation, and increased microsomal triglyceride transfer protein in a fructose-fed hamster model. J Biol Chem 2000; 275: 8416-8425.

46. Lewis GF, Uffelman KD, Szeto LW, Weller B, Steiner G. Interaction between free fatty acids and insulin in the acute control of very low density lipoprotein production in humans. J Clin Invest 1995; 95: 158-66.

47. Goldberg IJ. Lipoprotein lipase and lipolysis: central roles in lipoprotein metabolism and atherogenesis. J Lipid Res 1996; 37: 693-707.

48. De Man FH, Cabezas MC, Van Barlingen HH, Erkelens DW, de Bruin TW. Triglyceride-rich lipoproteins in non-insulin-dependent diabetes mellitus: post-prandial metabolism and relation to premature atherosclerosis. Eur J Clin Invest 1996; 26: 89-108.

49. Tavangar K, Murata Y, Pedersen ME, Goers JF, Hoffman AR, Kraemer FB. Regulation of lipoprotein lipase in the diabetic rat. J Clin Invest 1992; 90: 1672-8.

50. Semenkovich CF, Wims M, Noe L, Etienne J, Chan L. Insulin regulation of lipoprotein lipase activity in 3T3-L1 adipocytes is mediated at posttranscriptional and posttranslational levels. J Biol Chem 1989; 264: 9030-8.

51. Knutson VP. The release of lipoprotein lipase from 3T3-L1 adipocytes is regulated by microvessel endothelial cells in an insulin-dependent manner. Endocrinology 2000; 141: 693-701.

52. Yost TJ, Froyd KK, Jensen DR, Eckel RH. Change in skeletal muscle lipoprotein lipase activity in response to insulin/glucose in non-insulin-dependent diabetes mellitus. Metabolism 1995; 44: 786-90.

53. Bagdade JD, Kelley DE, Henry RR, Eckel RH, Ritter MC. Effects of multiple daily insulin injections and intraperitoneal insulin therapy on cholesteryl ester transfer and lipoprotein lipase activities in NIDDM. Diabetes 1997; 46: 414-20.

54. Rivellese AA, De Natale C, Di Marino L, Patti L, Iovine C, Coppola S, et al. Fasting hypertriglyceridemia in noninsulin-dependent diabetes mellitus is an important predictor of postprandial lipid and lipoprotein abnormalities. J Clin Endocrinol Metab 1991; 72: 934-44.

55. Howard BV. Insulin resistance and lipid metabolism. Am J Cardiol 1999; 84: 28J-32J.

56. Karpe F. Postprandial lipoprotein metabolism and atherosclersosis. J Intern Med 1999; 246: 341-55.

57. Ishida BY, Blanche PJ, Nichols AV, Yashar M, Paigen B. Effects of atherogenic diet consumption on lipoproteins in mouse strains C57BL/6 and C3H. J Lipid Res 1991; 32: 559-68.

58. Véniant MM, Pierotti V, Newland D, Cham CM, Sanan DA, Walzem RL, et al. Susceptibility to atherosclerosis in mice expressing exclusively apolipoprotein B48 or apolipoprotein B100. J Clin Invest 1997; 100: 180-8.

59. Ebara T, Conde K, Kako Y, Liu Y, Xu Y, Ramakrishnan R, et al. Delayed catabolism of apoB-48 lipoproteins due to decreased heparan sulfate proteoglycan production in diabetic mice. J Clin Invest 2000; 105: 1807-18.

60. Misra UK, Gawdi G, Gonzalez-Gronow M, Pizzo SV. Coordinate regulation of the α(2)-macroglobulin signaling receptor and the low

density lipoprotein receptor-related protein/α(2)-macroglobulin receptor by insulin. J Biol Chem 1999; 274: 25785-91.

61. Julien P, Vohl MC, Gaudet D, Gagne C, Levesque G, Despres JP, et al. Hyperinsulinemia and abdominal obesity affect the expression of hypertriglyceridemia in heterozygous familial lipoprotein lipase deficiency. Diabetes 1997; 46: 2063-8.

62. Gardner CD, Fortmann SP, Krauss RM. Association of small low-density lipoprotein particles with the incidence of coronary artery disease in men and women. JAMA 1996; 276: 875-81.

63. Lamarche B, Tchernof A, Mauriege P, Cantin B, Dagenais GR, Lupien PJ, et al. Fasting insulin and apolipoprotein B levels and low-density lipoprotein particle size as risk factors for ischemic heart disease. JAMA 1998; 279:1955-61.

64. Stampfer MJ, Krauss RM, Ma J, Blanche PJ, Holl LG, Sacks FM et al. A prospective study of triglyceride level, low-density lipoprotein particle diameter, and risk of myocardial infarction. JAMA 1996; 276: 882-8.

65. Rudel LL, Bond MG, Bullock BC. LDL heterogeneity and atherosclerosis in nonhuman primates. Ann NY Acad Sci 1985; 454: 248-53.

66. Jiang XC, Bruce C, Mar J, Lin M, Ji Y, Francone OL, et al. Targeted mutation of plasma phospholipid transfer protein gene markedly reduces high-density lipoprotein levels. J Clin Invest 1999; 103: 907-14.

67. Young SG, Fielding CJ. The ABCs of cholesterol efflux. Nat Genet 1999; 22: 316-8.

68. Arai T, Wang N, Bezouevski M, Welch C, Tall AR. Decreased atherosclerosis in heterozygous low density lipoprotein receptor-deficient mice expressing the scavenger receptor BI transgene. J Biol Chem 1999; 274: 2366-71.

69. Kozarsky KF, Donahee MH, Glick JM, Krieger M, Rader DJ. Gene transfer and hepatic overexpression of the HDL receptor SR-BI reduces atherosclerosis in the cholesterol-fed LDL receptor-deficient mouse. Arterioscler Thromb Vasc Biol 2000; 20: 721-7.

70. Clay MA, Newnham HH, Barter PJ. Hepatic lipase promotes a loss of apolipoprotein A-I from triglyceride-enriched human high density lipoproteins during incubation in vitro. Arterioscler Thromb 1991; 11: 415-22.

71. Haffner SM, Stern MP, Hazuda HP, Mitchell BD, Patterson JK. Cardiovascular risk factors in confirmed prediabetic individuals. Does the clock for coronary heart disease start ticking before the onset of clinical diabetes? JAMA 1990; 263: 2893-8.

72. Hansson L, Zanchetti A, Carruthers SG, Dahlöf B, Elmfeldt D, Julius S, et al. Effects of intensive blood-pressure lowering and low-dose aspirin in patients with hypertension: principal results of the Hypertension Optimal Treatment (HOT) randomised trial. HOT Study Group. Lancet 1998; 351: 1755-62.

73. UK Prospective Diabetes Study Group. Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38. Br Med J 2000; 317: 708-13.

74. Steiner G. Lipid intervention trials in diabetes. Diabetes Care 2000; 23(Suppl 2):B49-B53.

75. Srinivasan SR, Berenson GS. Bogalusa heart study. Am J Med Sci 1995; 310: S1-138.

76. Matsumoto T, Ohashi Y, Yamada N, Kikuchi M. Coronary heart disease mortality is actually low in diabetic Japanese by direct comparison with the Joslin cohort. Diabetes Care 1994; 17: 1062-3.

77. Aiello LP. Diabetic retinopathy (Technical Review). Diabetes Care. 1998; 21: 143-56.

78. UK Prospective Diabetes Study (UKPDS) Group: Effect of intensive blood glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). Lancet. 1998: 352: 854-65.

79. Maioli M, Tonolo G, Pacifico A, Ciccarese M, Brizzi P, Kohner EM, et al Raised serum apolipoprotein (a) in active diabetic retinopathy. Diabetologia 1993; 36(1): 88-90.

80. Heesen BJ, Wolffenbuttel BHR, Leurs PB, Sels JPJE, Menheere PPCA, Jäckle-Beckers SEC, et al. Lipoprotein(a) levels in relation to diabetic complications in patients with non-insuIin-dependent diabetes. Eur J Clin Invest 2008; 23 (9): 580-4.

81. Boemi M, Sirolla C, Fumelli P, James RW. Renal disease as a determinant of increased lipoprotein concentrations in diabetic patients. Diabetes Care 1999; 22 (12): 2033-6.

82. Fytili CI, Passadakis PS, Progia EG. Arterial hypertension is associated with serum lipoproteins (a) levels in end stage renal disease patients. Hemodial Int 2001; 5: 66-9.

83. Pickup J., Williams G. Text book of Diabetes: Clinical features of diabetic neuropathy. 2nd Ed. Oxford: Black Well Science; 1997.

84. Tseng CH. Lipoprotein (a) is an independent risk factor for peripheral arterial disease in Chinese type 2 diabetic patients in Taiwan. Diabetes Care 2004; 27(2): 517-21.

85. Nago N, Kayaba K, Hiraoka J, Matsuo H, Goto T, Kario K, et al. Lipoprotein (a) levels in the Japanese population: influence of age and sex, and relation to atherosclerotic risk factors. The Jichi Medical School Cohort Study. Am J Epidemiol 1995; 141(9): 815-21.

86. Jenner JL, Ordovas JM, Lamon-Fava S, Schaefer MM, Wilson PW, Castelli PW, et al. Effects of age, sex, and menopausal status on plasma lipoprotein(a) levels. The Framingham Offspring Study Circulation 1993; 87: 1135-41.

87. Enas EA, Yusuf S, Garg A, Davidson L, Thomas T, Pearson TA. Lipoprotein (a) levels in Indian physicians: comparison with white and black physicians in the USA. Indian Heart J 1994; 46:1

88. Bhatnagar D, Anand IS, Durrington PN, Patel DJ, Wander GS, Mackness MI, et al. Coronary risk factors in people from the Indian subcontinent living in West London and their sublings in India. Lancet 345: 405–409, 1995.

89. Sandholzer C, Hallman DM, Saha N, Sigurdsson G, Lackner C, Császár A, et al. Effects of the apolipoprotein(a) size polymorphism on the lipoprotein(a) concentration in 7 ethnic groups. Hum Genet. 1991; 86(6): 607-14.

90. Shaukat N, de Bono DP, Jones DR. Like father like son? Sons of patients of European or Indian origin with coronary artery disease reflect their parents' risk factor patterns. Br Heart J 1995; 74(3): 318–23.

91. Wilken DEL, Wang XLL, Dudman NTB. The APO A, B, a of coronary risk: back to kindergarten. Aust N Z J Med 1992; 22: 570-5.

92. Enas EA, Dhawan J, Petkar S. Coronary artery disease in Asian Indians: Lessons learned so far and the role of Lp(a). Ind Heart J 1997; 49: 25-34.

93. Dahle´n G. Lp (a) lipoprotein in cardiovascular disease. Atherosclerosis. 1994; 108: 111–26.

94. Emin K, Faruk O, Zeki A, Ramazan YM, Ali SR, Sami IS. Relationship Between Serum Lipoprotein (a) Levels and Retinopathy in Patients With Type 2 Diabetes. Annal Ophthalmol 2002; 34 (4): 198-203.

95. Kim CH, Park HJ, Park JY, Hong SK, Yoon YH, Lee KU. High serum lipoprotein (a) levels in Korean type 2 diabetic patients with proliferative diabetic retinopathy. Diabetes Care. 1998; 21(12): 2149-51.

96. Chopra R, Saramma JG, Mary J, Rebecca A. Lipoprotein (a) as a risk factor for diabetic retinopathy in patients with type 2 diabetes mellitus. Indian J Ophthalmol 2007; 55(3): 195-8.

97. Guillausseau PJ, Peynet J, Chanson P, Legrand A, Altman JJ, Poupon J, et al**.** Lipoprotein(a) in diabetic patients with and without chronic renal failure. Diabetes Care 1992; 15 (8): 976-9.

98. Boulton AJ, Malik RA, Arezzo JC, Sosenko JM. Diabetic Somatic Neuropathies. Diabetes Care 2004; 27(6): 1458-86.

99. Lakhotia M, Gehlot RS, Jain P, Sharma S, Singh M. Lipoprotein (a) in Type 2 Diabetic Subjects in relation to Diabetic Microvascular Complications. JIACM 2003; 4(4): 304-7.

100. Khare KC, Raman PG, Bhatnagar AD, Bhavsar R. Serum Lp(a) levels in patients of diabetes mellitus Int J Diab Dev countries 2000; 20: 79-83.

101. Maser RE, Usher DC, Decherney GS. Little association of lipid parameter and large sensory nerve fiber function in diabetes mellitus J Diab Comp 1996; 10: 54-9.

102. Angeline T, Aruna R, Ramadevi K, Mohan G, Jeyaraj N. Serum lipoprotein (a) and lipid profile in young South Indian patients with myocardial infarction. Ind J Clin Biochem 2003; 103-106.

103. Velmurugan K, Deepa R, Ravikumar R, Lawrence JB, Anshoo H, Senthilvelmurugan M, et al. Relationship of lipoprotein(a) with intimal medial thickness of the carotid artery in Type 2 diabetic patients in south India. Diabetic Medicine 2003; 2-(6): 455-61.

104. Mohan V, Deepa R, Haranath SP, Premalatha G, Rema M, Sastry NG, et al. Lipoprotein (a) is an independent risk factor for coronary artery disease in NIDDM patients in South India. Diabetes Care 1998; 21: 1819-23.

105. Luthra K, Vasisht S, Chhabra S, Raju KR, Agarwal DP, Manchanda SC, et al. LP (a) phenotypes and levels in angiographically proven coronary heart disease patients and controls. Ind J Clin Biochem 1998; 13(1): 12-9.

106. Maher VMG, Brown BG, Marcovina SM, Hillger LA, Xue-Qiao Z, Albers JJ. Effects of lowering elevated LDL cholesterol on the cardiovascular risk of lipoprotein (a) JAMA 1995; 274(22): 1771-4.

107. Tsimikas S, Brilakis ES, Miller ER, McConnell JP, Lennon RJ, Kornman KS, et al. Oxidized Phospholipids, Lp (a) Lipoprotein, and Coronary Artery Disease 2005; 353: 46-57.

108. Goldschmid MG, Barrett-Connor E, Edelstein SL, Wingard DL, Cohn BA, Herman WH. Dyslipidemia and ischemic heart disease mortality among men and women with diabetes. Circulation 1994; 89: 991-7.

109. Laakso M, Lehto S, Penttila I, Pyorala K. Lipids and lipoproteins predicting coronary heart disease mortality and morbidity in patients with non-insulin-dependent diabetes. Circulation 1993; 88: 1421-30.

110. Decossin C, Castro G, Derudas B, Bertrand M, Fruchart JC, Fiévet C. Subclasses of LpA-I in coronary artery disease: distribution and cholesterol efflux ability Eur J Clin Invest 1997; 27(4): 299-307.

111. Gomaraschi M, Sinagra G, Serdoz L, Pitzorno C, Fonda M, Cattin L, et al. The plasma concentration of Lpa-I: A-II particles as a predictor of the inflammatory response in patients with ST-elevation myocardial infarction. Atherosclerosis 2008; 12: 18486943 (P,S,G,E,B,D).

112. Shiffman D, Kane JP, Louie JZ, Arellano AR, Ross DA, Catanese JJ, et al. Analysis of 17,576 Potentially Functional SNPs in Three Case–Control Studies of Myocardial Infarction. Published online 2008; 6. doi: 10.1371/journal.pone.0002895.

113. Calmarza P, Cordero J, Santos V, Vella JC. Apolipoprotein (a) isoforms in infarcted men under 60 years old doi:10.1016/j.clinbiochem. 2003.06.001.

114. Bowden JF, Pritchard PH, Hill JS, Frohlich JJ. Lp(a) concentration and apo(a) isoform size. Relation to the presence of coronary artery disease in familial hypercholesterolemia. Arteriosclerosis and Thrombosis 1994; 14: 1561-8.

115. Wild SH, Fortmann SP, Marcovina SM. A prospective case-control study of lipoprotein (a) levels and apo (a) size and risk of coronary heart disease in Stanford Five-City project participants. Arteriosclerosis, Thrombosis, and Vascular Biology 1997; 17(2): 239-45.

116. Seman LJ, DeLuca C, Jenner JL, Cupples JA, McNamara JR, Wilson PWF, et al. Lipoprotein(a)-Cholesterol and Coronary Heart Disease in the Framingham Heart Study Clinical Chemistry 1999; 45: 1039-46.

117. Shai I, Schulze MB, Manson JE, Stampfer MJ, Rifai N, Hu FB. A prospective study of lipoprotein (a) and risk of coronary heart disease among women with type 2 diabetes. Diabetologia 2005; 48(8): 1469-76.

118. Rajappa M, Sridhar MG, Balachander J, Sethuraman KR. Lipoprotein (a) and comprehensive lipid tetrad index as a marker for coronary artery disease in NIDDM patients in South India. Clinica Chimica Acta 2006; 372(1-2): 70-5.

119. Nasri H. Association of Serum Lipoprotein (a) with Hypertension in Diabetic Patients. Saudi J Kidney Dis Transpl 2008; 19: 420-7.

120. Ariyo AA, Thach C, Tracy R. Cardiovascular Health Study Investigators Lp(a) Lipoprotein, Vascular Disease, and Mortality in the Elderly. N Engl J Med 2004; 350(11): 1150-2.

121. Lippi G, Caramaschi P, Montagnana M, Salvagno GL, Volpe A, Guidi G. Lipoprotein [a] and the lipid profile in patients with systemic sclerosis. Clinica Chimica Acta 2006; 364(1-2): 345-8.

122. Imperatore G, Rivellese A, Galasso R, Celentano E, Iovine C, Ferrara A et al. Lipoprotein (a) concentration in non-insulin-dependent diabetes mellitus and borderline hyperglycemia: a population-based study. Metabolism, Clinical and Experimental 1995; 44(10): 1293-7.

123. Habib SS, Aslam M, Naveed AK, Razi MS. Comparison of lipid profiles and lipoprotein (a) levels in patients with type 2 diabetes mellitus during oral hypoglycemic or insulin therapy. Saudi Med J 2006; 27: 174-80.

124. Rainwater DL, Haffner SM. Insulin and 2-hour glucose levels are inversely related to Lp (a) concentrations controlled for LPA genotype. Arterioscler Thromb Vasc Biol 1998; 18(8): 1335-41.

125. Simoes EJ, Kobau R, Kapp J, Waterman B, Mokdad A, Anderson L. Associations of physical activity and body mass index with activities of daily living in older adults. J Community Health 2006; 31(6): 453-67.

126. Akanji AO, Al-Shayji I, Kumar P. Metabolic and anthropometric determinants of serum Lp (a) concentrations and Apo (a) polymorphism in a healthy Arab population International Journal of Obesity 1999; 23 (8): 855-62.

ANNEXURE I – CONSENT FORM

Objective and purpose of the study

To study the correlation between serum lipoprotein (a) level and the development of vascular complications in type II diabetes mellitus patients.

The principal investigator of the study is **Dr. V. A. Kothiwale** **M.D, Ph.D** and the Co-investigator is **Dr. Preethy Verghese.**

Procedure

If I agree to be a part of the study, I will be asked the relevant history and will be subjected to relevant clinical examination. I will also undergo investigations like : Fasting Blood Sugar and Postprandial Blood Sugar, Fasting Lipoprotein (a), Fasting lipid profile, Complete blood count, ECG, 24 hours proteinuria, Urine routine and microscopy.

Risk and benefits

I have been explained by investigators, that there are no potential risks involved in this study and no benefits.

Alternatives

Taking part in this study is voluntary, I may choose not to take part in this study, or if I decide to take part I can later change my mind and withdraw from the study. My decision will not change the present or future health care or other services that I receive. The study doctor or sponsor may stop my participation in

this study anytime. If I choose not to take part in this study I will receive standard treatment for patients with my condition.

Privacy and confidentiality

All information collected about me during the course of this study will be kept confidential to the extent permitted by law. The code numbers will identify me in this research record.

Institutional / Sponsor policy

Does not apply to this research.

Financial incentives for participants

I will not be charged any amount for investigations subjected to me. I will not receive compensation or reimbursement for taking part in this study.

Authorization to publish results

Information from this study may be published but my identity will be confidential in any publication.

Consent statement

I voluntarily agree to take part in this study by signing below. I may withdraw at anytime. I am not giving up any legal rights by signing this form. My signature below indicates that I have read, or it has been read to me, this entire consent form, and have had all my questions answered.

Name of study participant or legally authorized representative:

Signature / Thumb print

In case of the queries during study or in future you may contact following person:

Principal Investigator :	Dr. V. A. Kothiwale.	Phone : 9448119899
Co-Investigator :	Dr. Preethy. Verghese	Phone : 9880349684
Name of the witness :		Signature :
Investigator Name :		Signature :
Date :		Place :

ANNEXURE II – PROFORMA

Case No.: OP. No.:

Name of the patient: Occupation:

Age: Sex: Male / Female

Socio economic status:

Residential address: If admitted D.O.A.

Phone No.: D.O.D.:

Clinical history

Duration:

Age of onset: Mode of onset:

How detected: Any previous

treatment:

Any other associated illness

Duration Treatment:

Any Specific complaints

Polyuria Polydipsia Polyphagia

Tiredness / Gen. Weakness Weight loss or

Weight gain

Headache Dizziness

Palpitation Dyspnoea

Vomiting Abdominal pain

Burning micturition Amenorrhoea

Tingling and numbness in extremities Loss of peripheral pulses

Vision : Short or long sight :

Cataract (Lt.) Eye (Rt.) Eye

Past History

Family History: Hypertension/ D.M. / I.H.D.

Personal History:

Diet : Veg. / Non-veg./Mixed

Appetite Sleep:

Bowels Micturitions : Day Night

Habits : Alcohol / smoking

General physical examination

Built : Obese / Lean / Average Nourishment:

Height : Weight: B.M.I. : Hip waist ratio

Pallor / Icterus / Cynanosis / Clubbing / Oedema

Gangrene of feet/ toes / fingers

Lymphadenopathy

Skin xanthomatous nodules: Grade of diabetic retinopathy changes**:**

Cardiovascular System:

Pulse	Rate	Rhythm	Volume	Character
Carotid				
Radial				
Femoral				
Popliteal				
Dorsalis pedis				

Blood pressure : **Resp. rate** **Temp.**

Systems	CVS	RS	GIT
Inspection			
Palpation			
Percussion			
Auscultation			

Central Nervous System:

Higher mental function	
Cranial nerves	
Power	
Tone	
Nutrition	
Sensory system	
D.T.R.	
Gait	

Investigations:

FBS	PPBS	
Lipoprotein (a) level	Serum cholesterol	
Serum Triglycerides	LDL	HDL
CBC		
ECG		
Urine routine		
Special Investigation.		

MASTER CHART

Sr. No.	IP. No.	Dem		Duration of DM (Years)	Family history of DM	Hypertension	Polyuria	Polydipsia	Polyphagia	Generalised weakness	Weight loss	Palpitation	Vomiting	Abdominal pain	Retinopathy	Serum creatimine (mg/dL)	Effective creatinine clearance (mg/dL)	Peripheral Neuropathy	Peripheral pulses	Total Cholesterol (mg/dL)	TGA (mg/dL)	LDL (mg/dL)	HDL (mg/dL)	Lp (a) (mg/dL)	FBS (mg/dL)	PPBS (mg/dL)	HbA1C (%)	WHR	BMI
		Age (Years)	Sex																										
1	206590	40	M	12	-	-	+	-	+	+	WL	+	+	+	+	1.6	55.56	+	-	316	373	180	32	43	419	512	15.5	0.96	22.6
2	206632	61	M	17	+	+	+	+	+	+	-	+	-	+	+	1.5	58	+	-	270	392	188	30	36	190	269	12.2	1.12	26.9
3	206641	43	M	17	-	+	+	+	+	+	WL	-	+	+	+	1.4	47.22	-	-	213	280	170	35	28	186	241	9	1.06	34.6
4	206649	50	M	12	+	+	+	+	+	+	WG	+	-	-	-	1.2	81.25	-	-	159	284	105	36	16.6	141	201	6.16	1.04	29.1
5	233317	58	M	13	-	+	+	-	+	+	WG	-	-	-	+	1.7	54.93	+	-	272	393	190	30	31	268	250	10.6	1.05	27.15
6	233324	68	M	14	+	-	+	+	+	+	-	+	-	+	+	1.9	34.74	+	-	158	219	79	35	26	212	247	9.4	1.15	27.5
7	237007	66	M	14	+	-	+	-	+	+	-	-	-	-	+	1.4	48.45	+	-	242	168	175	32	38	258	348	11.6	1.05	25.19
8	239531	66	M	12	-	-	+	-	+	+	-	-	-	-	+	1.5	39.74	+	-	260	352	243	21	33	216	240	8	0.92	21.88
9	239658	68	M	13	+	-	+	+	+	+	WG	-	-	-	+	1.9	38.95	+	-	189	167	122	34	80	166	308	6.9	1.02	27.61
10	239668	52	M	11	+	-	+	-	+	+	-	-	+	+	+	1.8	72	+	-	194	349	90	34	14	114	206	8	1.06	25.51
11	240231	64	M	11	-	-	+	+	+	+	WG	-	+	+	+	1.3	60.09	+	-	256	396	201	19	37	200	274	10.8	1.05	25.3
12	241068	68	M	13	-	-	+	-	+	+	-	-	-	-	+	1.6	40	+	-	108	179	158	34	27	117	200	8	1.08	25
13	241079	65	M	14	+	-	+	-	+	+	-	-	+	-	+	1.4	56.55	+	-	189	280	111	22	36	220	355	10.2	1.09	26
14	241081	56	M	10	-	-	+	-	+	+	-	-	+	-	+	1.5	62.22	+	-	242	391	129	35	38	236	320	8.3	0.93	25.3
15	241081	52	M	12	-	+	+	-	+	+	WG	+	+	+	+	1.5	59.48	-	-	196	276	156	36	10.2	126	200	7.2	1.06	25.2
16	241537	51	M	11	-	-	+	+	+	+	WG	-	-	-	+	1.5	57.69	-	-	183	119	127	32	42	230	296	8.8	1.13	23.4
17	244186	65	M	17	-	+	+	+	+	+	WG	-	-	-	+	1.3	21	+	-	159	269	99	27	50	261	500	9.6	0.91	23.6
18	244623	50	M	13	-	-	+	+	+	+	WG	+	+	+	+	1.4	64.29	+	-	218	221	139	35	36	196	216	8.6	1.12	24.9
19	245825	64	M	11	-	-	+	+	+	+	WG	-	-	-	+	1.5	47.85	+	-	200	192	129	33	42	188	248	9.2	0.86	22
20	247595	45	M	16	-	+	+	-	+	+	-	-	-	-	+	1.9	32.31	+	-	212	198	156	22	28	200	275	11	1.03	25
21	248540	67	M	11	-	-	+	-	+	+	WG	-	-	-	+	1.8	22	+	-	276	310	126	28	77.7	242	217	12.7	1.89	25.4
22	249671	65	M	13	-	-	+	-	+	+	-	-	-	-	+	1.4	52.08	+	-	192	469	176	33	39	216	268	9.6	1.06	23
23	249735	52	M	10	+	-	+	-	+	+	WG	+	+	-	+	1.6	51.94	-	-	194	113	133	38	33	240	315	10	1.07	24
24	251605	52	M	10	-	-	+	-	+	+	-	+	+	-	-	1.5	82	+	-	200	192	121	36	16	214	210	7	1.03	26.5
25	252649	58	M	11	+	+	+	+	+	+	-	-	-	-	-	1.5	80.28	+	-	216	359	107	37	32	221	288	8.8	0.95	23.5

MASTER CHART

Sr. No.	IP. No.	Dem		Duration of DM (Years)	Family history of DM	Hypertension	Polyuria	Polydipsia	Polyphagia	Generalised weakness	Weight loss	Palpitation	Vomiting	Abdominal pain	Retinopathy	Serum creatimine (mg/dL)	Effective creatinine clearance (mg/dL)	Peripheral Neuropathy	Peripheral pulses	Total Cholesterol (mg/dL)	TGA (mg/dL)	LDL (mg/dL)	HDL (mg/dL)	Lp (a) (mg/dL)	FBS (mg/dL)	PPBS (mg/dL)	HbA1C (%)	WHR	BMI
		Age (Years)	Sex																										
26	254494	55	M	11	-	-	+	-	+	+	-	+	+	-	+	1.9	59.03	+	-	220	278	163	30	26	180	279	10	0.48	29.4
27	254494	56	M	12	+	-	+	-	+	+	-	-	-	-	+	1.4	54.17	+	-	246	196	150	40	34	162	212	10	0.89	26.03
28	254494	54	M	10	+	+	+	-	+	+	-	+	-	-	+	1.2	77.64	+	-	286	192	156	20	20	170	300	10.8	0.94	29.1
29	254495	48	M	10	-	-	+	+	+	+	-	-	+	-	+	1.5	55.74	+	-	110	124	104	36	18	193	206	7.3	0.96	34.6
30	254501	47	F	10	-	+	+	-	+	+	-	-	-	-	+	1.1	50.76	-	-	261	312	88	47	10.2	349	262	10	0.84	21.4
31	256699	54	F	11	+	-	+	+	+	+	-	-	+	-	-	1.3	49.98	+	-	256	308	103	28	14.7	100	119	8	0.9	23.5
32	256863	60	F	16	-	+	+	-	+	+	-	-	+	-	+	1.3	49.4	+	-	256	380	160	32	28	184	221	10	1.02	27.9
33	258793	67	F	21	-	-	+	+	+	+	-	-	+	-	+	1.6	35.55	+	-	216	300	198	30	28	200	263	12.5	1.07	29.3
34	261870	55	F	13	-	-	+	+	+	+	-	-	-	-	+	1.8	33.45	+	-	170	294	130	16	31	196	280	7.6	1.07	25.29
35	261878	39	F	11	-	-	+	+	+	+	WG	-	-	-	-	1.6	41.17	-	-	161	149	84	47	6.8	136	218	7.6	0.75	23.66
36	262144	60	F	12	-	+	+	+	+	+	WG	+	-	-	+	1.6	37.78	+	-	118	127	70	22	26	160	231	6.8	0.84	25.7
37	262148	68	F	14	-	-	+	+	+	+	-	-	-	-	+	1.2	33	+	-	160	182	107	16	55.4	270	357	10	0.94	26.83
38	264025	48	F	11	-	-	+	+	+	+	-	-	-	-	+	1.9	38.87	+	-	246	200	136	22	28	204	276	9	0.88	28.6
39	264081	40	F	10	+	-	+	+	+	+	-	-	-	-	+	1.8	44.15	-	-	213	165	152	48	16	190	226	6	0.85	24.2
40	265631	50	F	10	+	-	+	+	+	+	-	+	-	-	+	1.4	47.05	-	-	188	162	123	33	15	126	220	8	0.82	24.8
41	284435	74	F	17	+	+	+	+	+	+	-	-	-	-	+	1.6	29.22	+	-	170	138	104	38	36	260	325	9.7	0.89	25.9
42	287706	68	F	18	-	+	+	+	+	+	-	-		-	+	1.2	48.17	+	-	189	137	122	40	24	196	238	8.4	0.93	24.7
43	288707	75	F	13	-	-	+	+	+	+	-	-	-	-	+	1.6	55	+	-	158	248	180	21	46	280	340	15.5	1.06	25.4
44	289573	60	F	13	-	-	+	+	+	+	-	-	-	-	+	1.6	30.69	+	-	280	180	140	23	31	200	250	11	0.9	20.25
45	289687	40	F	10	-	+	+	+	+	+	-	-	-	-	+	1.5	40.77	+	-	250	196	140	21	13	160	220	9.2	0.9	27.34
46	290402	58	F	13	-	-	+	+	+	+	-	-	-	-	+	1.6	39.84	+	-	256	200	180	24	17	250	312	9.8	0.95	25.9
47	290468	53	F	11	-	+	+	+	+	+	-	+	-	-	+	1.9	35.68	+	-	260	196	186	20	23	312	496	10	0.92	25.19
48	290573	61	F	16	-	-	+	+	+	+	-	-	-	-	+	1.8	34.2	+	-	260	196	134	24	15.7	206	312	11	0.9	29.3
49	290673	53	F	11	-	+	+	+	+	+	-	-	-	-	+	1.3	55.3	+	-	288	186	143	30	26	193	270	9.2	0.85	25.4
50	291546	65	F	17	-	-	+	+	+	+	-	-	-	-	+	1.6	34.31	+	-	280	188	130	21	42	162	290	12.5	0.88	26.82

ANNEXURE III – KEY TO MASTER CHART

Alb	-	Albumin
BMI	-	Body Mass Index
Dem	-	Demographic characteristics
DM	-	Diabetes Mellitus
FBS	-	Fasting blood sugar
HbA1C	-	Glycosylated haemoglobin
HDL	-	High density lipoprotein
I. P. No.	-	Inpatient number
LDL	-	Low density lipoprotein
Lp (a)	-	Lipoprotein (a)
mg/dL	-	Milligram per deci litre
NPDR	-	Non proliferative diabetic retinopathy
PDR	-	Proliferative diabetic retinopathy
PPBS	-	Post prandial blood sugar
Sr. No.	-	Serial number
TGA	-	Triglycerides
WG	-	Weight gain
WHR	-	Waist Hip Ratio
WL	-	Weight loss
Y	-	Years
+	-	Present
-	-	Absent