"A COMPARATIVE STUDY OF ASSOCIATION OF EXTENDED LIPID PROFILE WITH MICROVASCULAR COMPLICATIONS IN TYPE 2 DIABETES MELLITUS."

By

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In Partial Fulfilment Of The Requirements For The Degree Of

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IN

GENERAL MEDICINE

Under the guidance of

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I hereby declare that this dissertation entitled "**A COMPARATIVE STUDY OF ASSOCIATION OF EXTENDED LIPID PROFILE WITH MICROVASCULAR COMPLICATIONS IN TYPE 2 DIABETES MELLITUS.**" is a bonafide and genuine research work carried out by me under the guidance of **Dr. PRABHAKAR K., MD**, Professor and Head, Department of General Medicine, and Co-Guide **Dr. SHASHIDHAR.K.N**, Professor and Head, Department of Biochemistry, SDUMC, Kolar.

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ABSTRACT

A COMPARATIVE STUDY OF ASSOCIATION OF EXTENDED LIPID PROFILE WITH MICROVASCULAR COMPLICATIONS IN TYPE 2 DIABETES MELLITUS.

BACKGROUND:

Type 2 Diabetes Mellitus is one of the common medical illness and serious metabolic disorder which is rapidly raising pandemic in Indian population with early onset than our western counterparts. Diabetes causes microvascular and macrovascular complications but microvascular complications like Diabetic Retinopathy, Diabetic Nephropathy, and diabetic Neuropathy are most common.

Diabetic dyslipidemia encompasses both quantitative and qualitative changes in plasma lipids and lipoproteins, characteristically observed in individuals with type-2 diabetes mellitus.

Study on the levels of lipoprotein (a), apolipoprotein A1 and apolipoprotein B and its association with microvascular complications in type 2 DM will bring to limelight the importance and potentiate the clinical application of extended lipid profile as an early marker for microvascular complication and early treatment.

OBJECTIVES OF THE STUDY:

1. To estimate the levels of extended lipid profile in type 2 diabetes mellitus patients and associate them with diabetic micro vascular complications.

2.To compare extended lipid profile with diabetic micro vascular complications and patients with diabetes but without microvascular complications

MATERIALS AND METHODS:

A hospital based cross sectional study consisting of 130 Type-2 Diabetes Mellitus of which 65 subjects were with microvascular complications and 65 subjects were without microvascular complications was taken into study and detailed diabetic history, personal history was taken and was investigated for glycemic status, regular lipid profile and novel extended lipid profile Lipoprotein (a), Apolipoprotein A1 and Apolipoprotein B. Novel extended lipid parameters estimation was done by immunoturbudometric metod. Value of for lipoprotein (a) >30 mg/dl, apolipoprotein A1 101-215 mg/dl, Apolipoprotein B 51-132 mg/dl was taken as normal and the two groups were compared.

RESULTS:

The mean age in diabetic patients with microvascular complication and without microvascular complication was 63.51±10.51 years and 57.60±11.82 respectively. Sex, BMI, habits were matched. The prevalence of diabetic nephropathy was higher than retinopathy and diabetic neuropathy had lesser incidence. Patients with microvascular complications had poor glycemic control $p<0.001$ compared to patients with microvascular complications. Mean values of lipid fractions like Lp (a), Apo A1, Apo B, serum total cholesterol, triglycerides, LDL and VLDL were higher in type2 diabetes with microvascular complications when compared to type-2 diabetes without microvascular complications p value 0.028.

CONCLUSION:

Patients with microvascular complications i.e retinopathy, nephropathy, and neuropathy had poor glycemic control with increased duration of diabetes. Diabetic dyslipidemia (hypertriglyceridemia, VLDL, LDL, decreased HDL) was more common and novel extended lipid parameters (Lp (a), Apo A1, Apo B) may be used as novel biomarkers for early detection of microvascular complications. Further studies are need to correlate and associate diabetic neuropathy and novel extended lipid parameters.

KEYWORDS:

Type 2 Diabetes Mellitus, Microvascular complications, Diabetic Retinopathy, Diabetic Nephropathy, Diabetic Neuropathy, Total cholesterol, HDL, LDL, VLDL, Lipoprotein (a), Apolipoprotein A1, Apolipoprotein B.

ABBREVATIONS

AAO	American Association of Ophthalmology
ACC	American College of Cardiology
ACE	Angiotensin Converting Enzyme
ADA	American Diabetes Association
AER	Albumin Excretion Ratio
AGE	Advanced Glycation End product
Apo (a)	Apolipoprotein (a)
Apo A1	Apolipoprotein A1
Apo B	Apolipoprotein B
BMI	Body Mass Index
CAD	Coronary artery disease
CETP	Cholesteryl Ester Transfer Protein
CVD	Coronary vascular disease
DM	Diabetes mellitus
DME	Diabetic Macular Edema
ECM	Extra Cellular Matrix
ESRD	End Stage Renal Disease
E.g	Example
Ex	Examination

F/H	Family History
FBS	Fasting Blood Sugars
GBM	Glomerular Basement Membrane
GFR	Glomerular Filtration Rate
Gr	Grade
HbA1C	Glycated Hemoglobin
HDL	High density lipoprotein cholesterol
HRT	Hormone Replacement Therapy
HTN	Hypertension
IDDM	Insulin Dependent DM
IDL	Intermittent density lipoprotein
i.e	that is
LCAT	Lecithin Cholesterol Acyl Transferase
LDL	Low density lipoprotein cholesterol
Lp (a)	Lipoprotein (a)
LVF	Left Ventricular Failure
MA	Micro Albuminuria
mRNA	messenger ribonucleic acid
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NCS	Nerve Conduction Study

NIDDM	Non-Insulin Dependent Diabetes mellitus
NPDR	Non Proliferative Diabetic Retinopathy
OAD	Oral Anti Diabetic
PDR	Proliferative Diabetic Retinopathy
PKC	Protein Kinase C
PPBS	Post Prandial Blood Sugar
PVD	Peripheral Vascular Disease
RBC	Red Blood Cell
RBS	Random Blood Sugar
ROS	Reactive Oxygen Species
SDH	Sorbitol Dehydrogenase
TC	Total cholesterol
TG	Triglycerides
UKPDS	United Kingdom Prospective Diabetes Studies
VGEF	Vascular Endothelial Growth Factors
VLDL	Very Low Density Lipoprotein
WHO	World Health Organization

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INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels[1,2].

The importance of protecting the body from hyperglycaemia cannot be overstated; the direct and indirect effects on the human vascular tree are the major source of morbidity and mortality in both type 1 and type 2 diabetes. Generally, the injurious effects of hyperglycaemia are separated into macrovascular complications (coronary artery disease, peripheral arterial disease, and stroke) and microvascular complications (diabetic nephropathy, neuropathy, and retinopathy). It is important to understand the relationship between diabetes and vascular disease because the prevalence of diabetes continues to increase[3].

Type 2 Diabetes Mellitus is associated with dyslipidaemia comprising of multiple lipoprotein disorders. Dyslipidemia is one of the major risk factors for cardiovascular disease in diabetes mellitus. The characteristic features of diabetic dyslipidemia are high plasma triglyceride, low High density lipoprotein (HDL) cholesterol and increased small dense low density lipoprotein (LDL)-cholesterol particles[4].

Extended lipid profile comprises of estimation of classical lipid profile, lipoprotein (a), and Apolipoprotein A1 and Apolipoprotein B estimation.

Lipoprotein (a), Apo lipoprotein A and Apo lipoprotein B are important independent and inheritable risk factor for micro/ macro vascular complications in diabetics. Lipoprotein (a) is also a risk factor for the progression of diabetic nephropathy with overt

proteinuria[5,6].High serum Apo lipoprotein have been detected in patients with diabetic retinopathy, nephropathy and neuropathy.

The relationship between the diabetic microvascular complication and dyslipidemia including extended lipid profile(viz, lipoprotein (a), apolipoprotein A1 and Apolipoprotein B is now of special interest as these have been found to be novel atherosclerotic risk factors in diabetic microvascular complication in world and India which have common link between traditional lipids and extended lipid profile.

AIMS AND OBJECTIVES OF THE STUDY

1. To estimate the levels of extended lipid profile in type 2 diabetes mellitus patients and associate them with diabetic micro vascular complications.
2. To compare extended lipid profile with diabetic micro vascular complications and patients with diabetes but without microvascular complications

HISTORICAL ASPECTS

Diabetes Mellitus

The knowledge of diabetes dates back to centuries before Christ. Polyuric state, resembling diabetes was described as early as 150 BC by the Egyptian Papyrus Ebers.

Celsus (30 BC-50 AD) had recognized the disease.The term diabetes which is Greek and means "run thro" or 'siphon'[7] was first was first described by Aretaeus in second century AD as generic description for condition causing increased urine output. Roman Physicians Celsus and Aretaeus who lived in the first century (30-90 AD) described diabetes as a "wonderful affection, not very frequent among men, being melting of flesh and limbs into urine"[8].

The association of polyuria with a sweet tasting substance in urine – Madhumeha was first reported in Sanskrit literature dating from 5^{th} to 6^{th} century AD at that time of two noted surgeon and physician Shushrutha and Charaka.

It was in 17^{th} century that Thomas Willis (1621-1675) made the observation as if imbibed with honey and sugar about the diabetic urine.

A century after Willis, Mathew Dobson (1735-1784) demonstrated that sweetness of urine was indeed due to sugars.

John Rollo was one of the first to use adjective mellitus (mellitus = honey) to distinguish from other polyuric states in which urine was tasteless (Insipidus in Greek).

The diabetes was over whelmed with joy in 1921 when young physician and surgeon , Fedrick Grant Banting (1891-1941) and Charles H Best, his graduate student assistant , working in Toranto , prepared active extracts of pancreas which lowered the elevated level of

sugar in diabetic dogs. The first patient to be treated with pancreatic extract was Leonard Thomson who was treated in January 1922.

DIABETIC RETINOPATHY[9]

In 1846, the French ophthalmologist and Professor of Hygiene in Paris, Appolinaire Bouchardat (1806-1886), reported the development of visual loss in the absence of cataract in diabetics.

Diabetic macular changes in the form of yellowish spots and full or partial thickness extravasations through the retina were observed for the first time by Eduard Jäger. In 1855, he published "Beiträge zur Pathologie des Auges" where he included his fundus paintings.

In 1872, Edward Nettleship published his seminal paper on "Oedema or cystic disease of the retina", providing the first histopathological proof of "cystoid degeneration of the macula" in patients with diabetes.

In 1876, Wilhelm Manz (1833-1911) published his seminal paper on 'Retinitis proliferans' containing several drawings of fibrovascular degeneration of the optic disc and vitreoretinal adhesions in the retina.

In 1890, Julius Hirschberg (1843-1925) classified diabetic retinopathy into four types (retinitis centralis punctuate, haemorrhagic form, retinal infarction, and haemorrhagic glaucoma) describing the full natural history of diabetic retinopathy.

DIABETIC NEPHROPATHY

The Diabetic nephropathy was discovered by British physician Clifford Wilson and Germany-born American physician Paul Kimmelstiel and was published for the first time in 1936.

DIABETIC NEUROPATHY

Although the recognition of DM dates back to several centuries ago the association between diabetes and pheripheral nerve disorder was not recognized until a century ago.

The earliest reference to symptoms related to neuropathy are traceable to the writings of Jhon Rollo (1778) who a patient with pain and paraesthesia in the legs. Later Claude Bernard (1885) with documentation of hyperglycaemia following a puncture of the 4^{th} ventricle, considered DM to be a result of disturbances in the CNS. But Marchal de calvi (1864) was the first to suggest that neurological disorder noted in diabetes could be a consequence rather than cause of the disease. Marchal demonstrated paraesthesias and shooting pains and recognized that in some patients these may be the presenting symptoms of diabetes.

The loss of tendon reflexes in legs was described by Bouchard[10] and occurrence of spontaneous pains and hyperesthesia by Pavy[11] (1885). Motor manifestations in the legs were documented in 1890 by Buzzard, Burns and Charcot, cranial nerve involvement by Ogle and autonomic neuropathy by Jordan (1936), Rundles (1945).

DYLIPIDEMA

Boas and associates found frequent association of hypercholesterolemia and coronary atherosclerosis in 1940. In 1941, Blix et al, electrophoretically separated lipoproteins into Total Cholesterol, Triglycerides, VLDL, HDL.

In the year 1949 Goffman discovered entire spectrum of lipoproteins based on variation in floatation rate. In 1967, Donald S Fredrickson gave the classification of hyperlipidaemia and hyperlipoproteinaemia. High-density lipoprotein was first isolated by Scann in the year 1968

TYPE 2 DIABETES MELLITUS

EPIDEMIOLOGY

Type-2 diabetes is a major health problem all over the world. It is the commonest form comprising 90 to 95% of all the diabetic population in any country[12, 13].

Globally, an estimated 422 million adults are living with diabetes mellitus, according to the latest 2016 data from the World Health Organization (WHO).⁻Diabetes prevalence is increasing rapidly; previous 2013 estimates from the International Diabetes Federation put the number at 381 million people having diabetes. The number is projected to almost double by 2030[14].

Diabetes currently affects more than 62 million Indians, which is more than 7.1% of the adult population. The average age on onset is 42.5 years. Nearly 1 million Indians die due to diabetes every year[15].

According to the Indian Heart Association, India is projected to be home to 109 million individuals with diabetes by 2035. A study by the American Diabetes Association reports that India will see the greatest increase in people diagnosed with diabetes by 2030[16].

Classification of Diabetes[17]:

Diabetes can be classified into:

1. Type 1 diabetes (due to b-cell destruction, usually leading to absolute insulin deficiency)

2. Type 2 diabetes (due to a progressive loss of insulin secretion on the background of insulin resistance)

3. Gestational diabetes mellitus (GDM) (diabetes diagnosed in the second or third trimester of pregnancy that is not clearly overt diabetes)

4. Specific types of diabetes due to other causes, e.g., monogenic diabetes syndromes (such as neonatal diabetes and maturity-onset diabetes of the young [MODY]), diseases of the exocrine pancreas (such as cystic fibrosis), and drug- or chemical-induced diabetes (such as with glucocorticoid use, in the treatment of HIV/AIDS or after organ transplantation)

Criteria for the diagnosis of diabetes[17]:

American Diabetes Association - 2016

HbA1C ≥6.5%. or

FPG ≥126 mg/dL, Fasting is defined as no caloric intake for at least 8 hours

2-h PG ≥200 mg/dL, during an OGTT or

In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥200 mg/dL.

Risk factors associated with Type 2 Diabetes[18]:

- Family history of diabetes mellitus
- Obesity (BM1 ≥ 25 kg/m^2)
- Habitual physical inactivity
- Race/ethnicity
- Previously identified IFG or IGT or haemoglobin A_{1c} of 5.7-6.4%

- History of gestational diabetes mellitus or delivery of baby weighing ≥ 4 kg.
- Hypertension (Blood pressure ≥ 140/90 mmHg)
- HDL cholesterol< 35 mg/dl and /or Triglyceride level ≥ 250mg/dl
- Polycystic ovarian disease
- History of cardiovascular disease

SMOKING AND DIABETES[19]

Smoking is one of the modifiable risk factors for many chronic diseases, such as cardiovascular disease (CVD), cancer, chronic obstructive lung disease, asthma, and diabetes. Many studies have shown that the adverse effects of smoking on diabetes mellitus are not only diabetic macrovascular complications but the causal nature of its association with diabetes and the progression of diabetic microvascular complications has yet to be explored. Smoking increases inflammation and oxidative stress, which directly damage β-cell function and impair endothelial function.

ALCOHOL AND DIABETES[20]

Chronic use of alcohol is a potential risk factor for the incidence of type 2 diabetes mellitus (T2DM), which causes insulin resistance and pancreatic β-cell dysfunction.

There is evidence that chronic heavy consumption of alcohol has deleterious effect on metabolic control and associated with impaired insulin resistance. The pancreatic islet β-cells normally increase the insulin release sufficiently to overcome the reduced efficiency of insulin action. Thereby, maintaining normal glucose tolerance. However, chronic heavy alcohol use leads to impaired glucose tolerance, which is a combination of impaired secretion of insulin and a reduced insulin sensitivity or resistance. Glucose intolerance is the transition phase between normal glucose tolerance and diabetes, also referred to as prediabetes.

In alcoholic patients, increased insulin resistance which is associated with chronic pancreatitis or insulin secretion is associated with alcohol-induced liver changes. High concentrations of ethanol lead to reduced insulin binding and inhibition of intracellular signaling related to insulin.

Appetite-regulating peptides, particularly ghrelin and leptin, BDNF, and hippocampal LTP, play important roles in the brain and insulin sensitivity, and are possible mechanism for mediation that links T2DM and alcohol consumption. The novel mechanisms of these two appetite regulating peptides, BDNF and hippocampal LTP are widely involved in the neurobiology of alcohol dependence and T2DM.

Complications of Type-2 Diabetes[21]:

Micro vascular

• Eye disease

-Retinopathy (nonproliferative/proliferative)

-Macular edema

• Neuropathy

-Sensory and motor (mono- and polyneuropathy)

-Autonomic

• Nephropathy (albuminuria and declining renal function)

Macro vascular

- Coronary heart disease
- Peripheral vascular disease
- Cerebrovascular disease

Other

- Gastrointestinal (gastro paresis, diarrhea)
- Genitourinary (uropathy/sexual dysfunction)
- Dermatological
- Infectious
- Cataract and Glaucoma
- Cheiroarthropathy
- Periodontal disease
- Hearing loss

DIABETIC RETINOPATHY

Diabetes is now regarded as an epidemic, with the population of patients expected to rise to 380 million by 2025. Tragically, this will lead to approximately 4 million people around the world losing their sight from diabetic retinopathy, the leading cause of blindness in patients aged 20 to 74 years[22].

DEFINITION

Diabetic retinopathy is a chronic progressive, potentially sight-threatening disease of the retinal microvasculature associated with the prolonged hyperglycaemia and other conditions linked to diabetes mellitus such as hypertension.

NATURAL HISTORY OF DIABETIC RETINOPATHY

Diabetic retinopathy progresses from mild nonproliferative abnormalities, characterized by increased vascular permeability, to moderate and severe nonproliferative diabetic retinopathy (NPDR), characterized by vascular closure, to proliferative diabetic retinopathy (PDR), characterized by the growth of new blood vessels on the retina and posterior surface of the vitreous. Macular edema, characterized by retinal thickening from leaky blood vessels, can develop at all stages of retinopathy. Pregnancy, puberty, blood glucose control, hypertension, and cataract surgery can accelerate these changes.

Vision-threatening retinopathy is rare in type 1 diabetic patients in the first 3–5 years of diabetes or before puberty. During the next two decades, nearly all type 1 diabetic patients develop retinopathy. Up to 21% of patients with type 2 diabetes have retinopathy at the time

of first diagnosis of diabetes, and most develop some degree of retinopathy over time. Vision loss due to diabetic retinopathy results from several mechanisms. Central vision may be impaired by macular edema or capillary nonperfusion. New blood vessels of PDR and contraction of the accompanying fibrous tissue can distort the retina and lead to tractional retinal detachment, producing severe and often irreversible vision loss. In addition, the new blood vessels may bleed, adding the further complication of preretinal or vitreous haemorrhage. Finally, neovascular glaucoma associated with PDR can be a cause of visual loss[23.]

CLASSIFICATION [24, 25, 26]

Diabetic retinopathy is classified according to the presence or absence of abnormal new vessels as:

1. Non-proliferative (background/preproliferative) retinopathy
2. Proliferative retinopathy
3. Diabetic maculopathy (DM)

Non-proliferative diabetic retinopathy (NPDR) (background/preproliferative)

In the international (AAO) classification, NPDR is graded as: Mild, Moderate and Severe

Proliferative diabetic retinopathy (PDR)

PDR is described according to:

(a) Location

• New vessels on the disc (NVD) or within 1 disc diameter (DD) of the margin of the disc

• New vessels elsewhere in the retina (NVE) (more than 1DD from the disc)

(b) Severity

Early PDR, PDR with high risk characteristics, florid PDR and gliotic PDR.

Diabetic maculopathy (DM)

Retinopathy which affects the macula is separately described as diabetic maculopathy.

DM is further classified as: Focal oedema, diffuse oedema, ischaemic or mixed.

1. **Non-proliferative diabetic retinopathy**:

Retinal microvascular changes that occur in NPDR are confined to retina and do not extend beyond internal limiting membrane.

Characteristic changes in retina are

1. Microaneurysms
2. Areas of capillary nonperfusion
3. Nerve fibre layer infarcts (called cotton-wool spots)
4. Intraretinal microvascular abnormalities (IRMA's)
5. Dot and blot intraretinal haemorrhages
6. Retinal oedema
7. Hard exudates
8. Arteriolar abnormalities
9. Dilatation and beading of retinal veins

Visual loss in this stage occurs because of 2 mechanisms.

1. Increased intraretinal vascular permeability, resulting in macular oedema
2. Variable degrees of intraretinal capillary closure

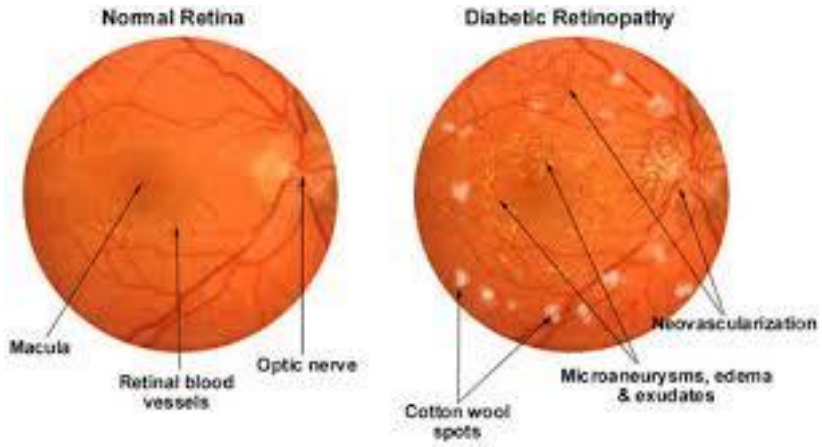


Fig 1: Normal retina and non proliferative diabetic retinopathy.

Courtesy:arleoeye.com

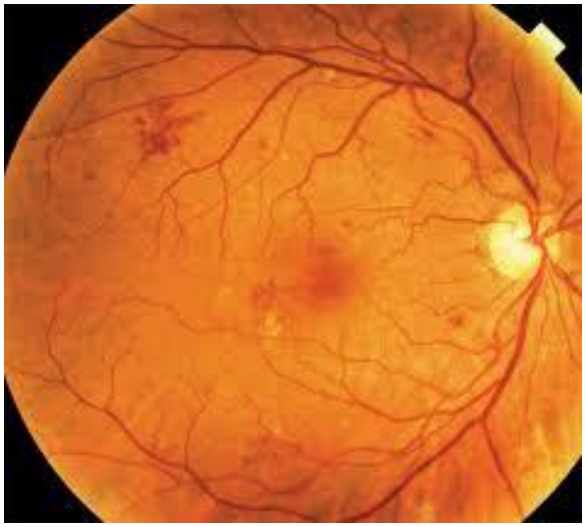


Fig 2: Fundus picture of non proliferative diabetic retinopathy.

Courtesy: Phoenixvillieyecare.com

Diabetic macular edema

The diagnosis of DME is made when retinal thickening is noted by slit lamp biomicroscopy

Findings are

1. Location of retinal thickening relative to the foveal center
2. Presence and location of exudates
3. Presence of csystoid macular oedema

Macular oedema can manifest as focal macular edema or diffuse macular edema.

Focal macular edema

Is characterized by areas of focal fluorescein leakage from capillary lesions, such as microaneurysms.

Diffuse macular edema

Is characterized by wide spread retinal capillary leakage and extensive break down of blood-retinal barrier, often with cystoid fluid accumulation in the pre foveal macula.

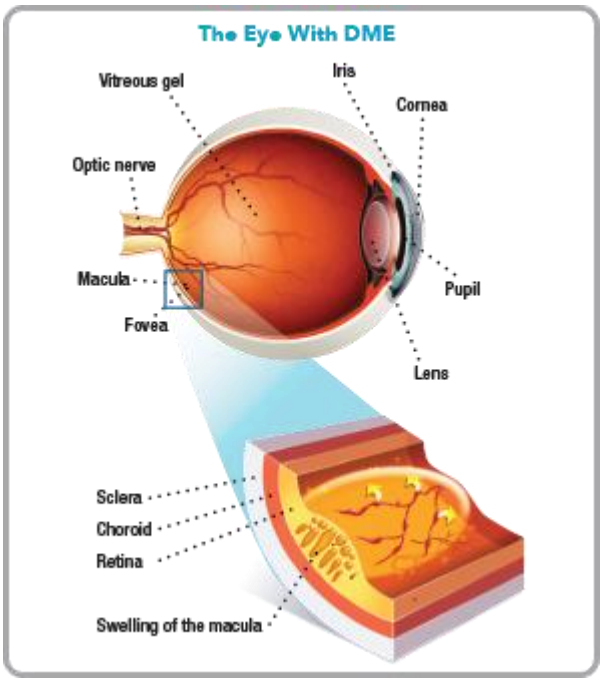


Fig 3: Diagrammatic representation of diabetic macular oedema.

Courtesy: Orudex.com

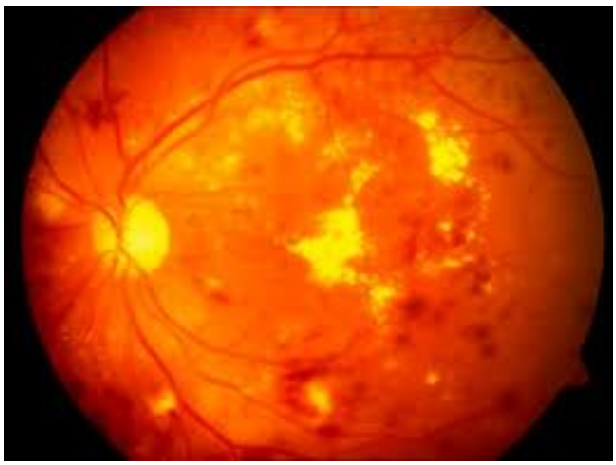


Fig 4: Fundus picture of diabetic macular oedema.

Courtesy: drdhanashreesta.blogspot.com

Proliferative diabetic retinopathy:

Extraretinal fibrovascular proliferation is present in varying stages of development in PDP.

The new vessels evolve through 3 stages

1. Fine new vessels with minimal fibrous tissue cross and extend beyond Internal Limiting membrane.
2. The new vessels increase in size and extent, with an increased fibrous component.
3. The new vessels regress, leaving residual fibrovascular tissue that uses posterior hyaloid as a scaffold.

Based on the extent of proliferation, PDR is graded into early, high-risk or advanced.

High risk PDR is defined as presence of any of the following findings

1. Presence of vitreous or prereitinal haemorrhage
2. Presence of new vessels
3. Location of new vessels on or near the optic disc
4. Moderate to severe extent of new vessels

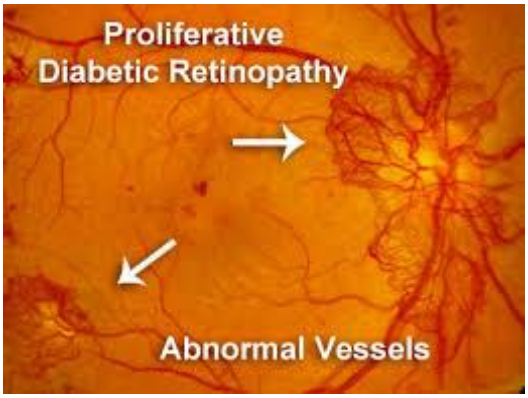


Fig 5: Fundus picture of proliferative diabetic retinopathy.

Courtesy: Retina vitreous foundation of Florida

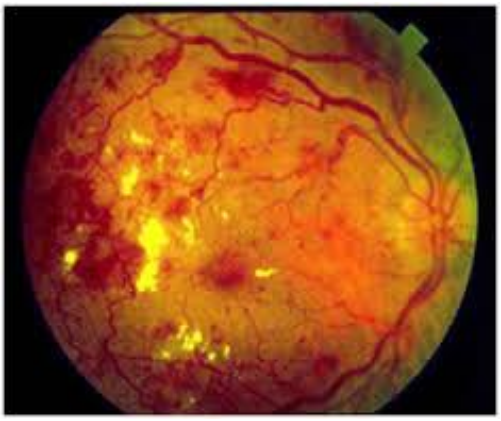


Fig 6: Funds picture of proliferative diabetic retinopathy 2.

Courtesy: Stone Oak Ophthalmology

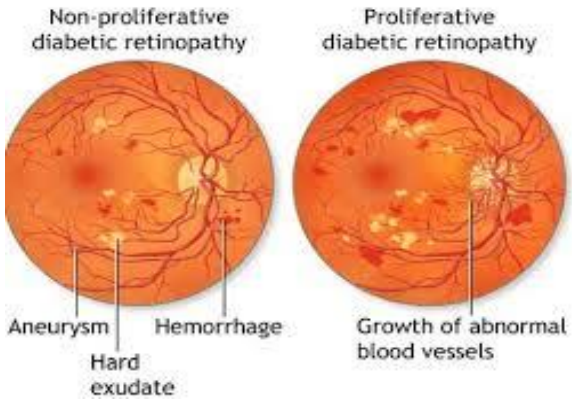


Fig 7: Showing difference between non proliferative and proliferative diabetic retinopathy.

Courtesy: New health guide

PATHOGENISIS AND PATHOPHYSIOLOGY

Several biochemical pathways that have been proposed and tested as key contributors in the development of DR[22]

1. Increased polyol pathway flux
2. Activation of protein kinase C (PKC)
3. Increased expression of growth factors such as vascular endothelial growth factor (VEGF) and insulin-like growth factor-1 (IGF-1)
4. Haemodynamic changes
5. Accelerated formation of advanced glycation end products (AGEs)
6. Oxidative stress
7. Activation of the renin-angiotensin-aldosterone system (RAAS)
8. Subclinical inflammation and leukostasis
9. Capillary occlusion
10. Retinal neurodegeneration

Some of the important mechanisms are

INCREASED POLYOL PATHWAY FLUX

In diabetes, the polyol pathway metabolises excess glucose. The enzyme aldose reductase (AR) present in the retina reduces glucose into sorbitol using nicotinamide adenine dinucleotide phosphate (NADPH) as a cofactor. Sorbitol is subsequently converted into fructose by sorbitol dehydrogenase (SDH). Since sorbitol is impermeable to cellular membranes, it accumulates within the cell, and this is followed by the slow metabolism of

sorbitol to fructose. NADPH is also required for glutathione reductase as a cofactor for regenerating intracellular glutathione in cells, thus reducing the antioxidant capacity of the cells. The build-up of sorbitol is thought to have multiple damaging effects in retinal cells including osmotic damage[22].

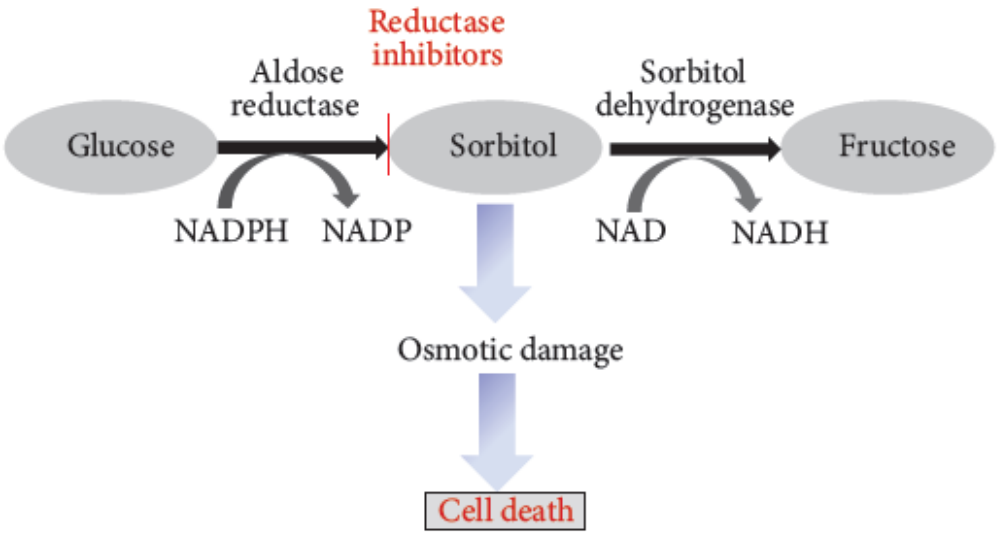


Fig 8: Polyol pathway in diabetic retinopathy.

From Joanna M et al Review article

Recent studies have demonstrated that increased AR is localised in several retinal cells including pericytes, retinal endothelial cells, ganglion cells, Müller cells, retinal pigment epithelial cell, and neurons[27, 28]. Studies also demonstrate that increased AR activity is involved in the destruction of retinal cells. Exposure of pericytes or endothelial cells to increased concentrations of glucose or galactose resulted in reduced viability of cells. However, this cell death was reversed upon the addition of ARIs[29, 30].

PROTEIN KINASE C (PKC) ACTIVATION

Protein kinase C (PKC) is a family of 10 enzymes, in which the $\beta\beta$1/2 isoform appears to be closely associated with the development of diabetic retinopathy[31]. Hyperglycemia increases de novo synthesis of diacylglycerol, which is an activating factor for the isoforms of protein kinase C. This activation in turn regulates various pathophysiological processes. The expression of the PKC $\beta\beta$1/2 isoform is enhanced in patients with diabetes. Since PKC is involved in a number of physiological processes, its upregulation contributes to the pathogenesis of diabetic retinopathy in the form of differential synthesis of extracellular matrix (ECM) proteins and ECM remodelling, enhanced release of angiogenic factors, endothelial and leukocyte dysfunction leading to capillary occlusion and leukostasis, and changes in blood flow to the retina. As a result the PKC pathway directly influences other pathways such as inflammation, neovascularisation, and aberration of haemodynamic, which in turn further contribute to the pathogenesis and progression of diabetic Retinopathy[32].

INCREASED EXPRESSION OF GROWTH FACTORS SUCH AS VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) AND INSULIN-LIKE GROWTH FACTOR-1 (IGF-1)

The involvement of growth factors in diabetic retinopathy is supported by the clinical evidence of the development of retinopathy during puberty and the observation that serious retinopathy is rarely observed in growth hormone deficient diabetic dwarfs[33]. There are a number of growth factors which have been associated with the development of diabetic retinopathy and

these include basic fibroblast growth factor (bFGF)[34], insulin-like growth factor-1 (IGF-1), angiopoietin- 1 and -2 , stromal-derived factor-1, epidermal growth factor (EGF) , transforming growth factor-beta 2 (TGF-$\beta\beta$2) , platelet-derived growth factors (PDGFs) , and erythropoietin .

The growth factor which is the most widely studied in relation to diabetic retinopathy is VEGF which exists infour homodimer molecular species, each monomer having, respectively, 121, 165, 189, or 206 amino acids[35]. VEGF promotes angiogenesis; causes breakdown of the blood retinal barrier, stimulation of endothelial cell growth, and neovascularisation; and increases vascular permeability in the ischemic retina[36].

The cellular functions of VEGF are mediated by the activation of two membrane bound tyrosine kinase receptors[37]. The binding of VEGF to the membrane bound receptors activates two possible pathways, a calcium influx channel or a mitogen activating protein kinase signalling pathway. Both pathways lead to the vascular leakage and blood retinal barrier breakdown with which VEGF has been associated. The angiogenic role in the retina to which VEGF has been linked is thought to be due to an interaction with angiotensin II[38].

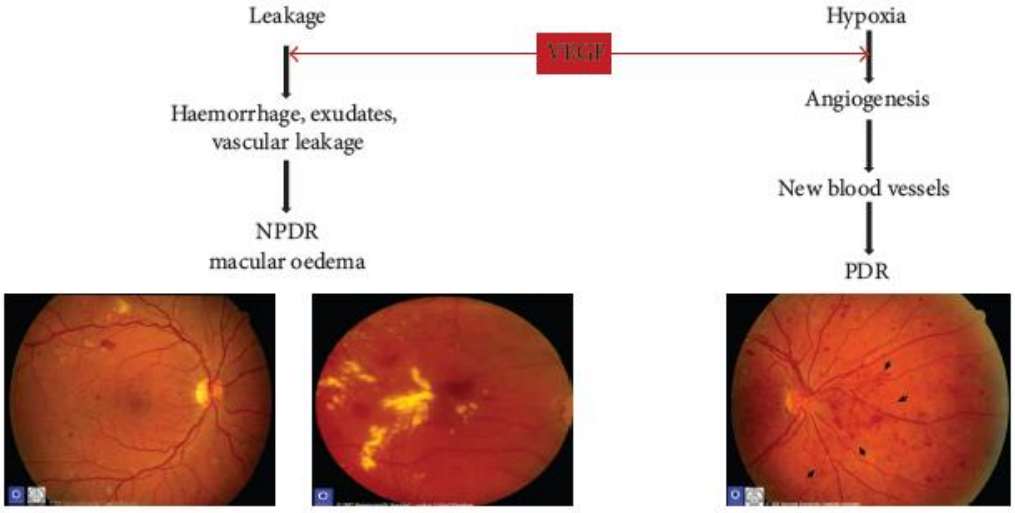


Fig 9: Vascular endothelial growth factor (VEGF) pathways in nonproliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR) and carbonic anhydrase (CA).

(From Joanna M et al Review article)

ACCELERATED FORMATION OF ADVANCED GLYCATION END PRODUCTS (AGES)

Among the several pathogenic mechanisms that may contribute to diabetic retinopathy are the formation and accumulation of AGEs[39]. AGEs are a heterogeneous group of molecules formed from the nonenzymatic reaction of reducing sugars with free amino groups of proteins, lipids, and nucleic acids. The initial product of this reaction is called a Schiff base, which spontaneously rearranges itself into an Amadori product[40].

AGEs affect cells by three main mechanisms: (1) as adducts occurring on modified serum proteins, (2) as endogenous adducts formed as a consequence of glucose metabolism, or as extracellular matrix-immobilised modifications of long-lived structural proteins[41].

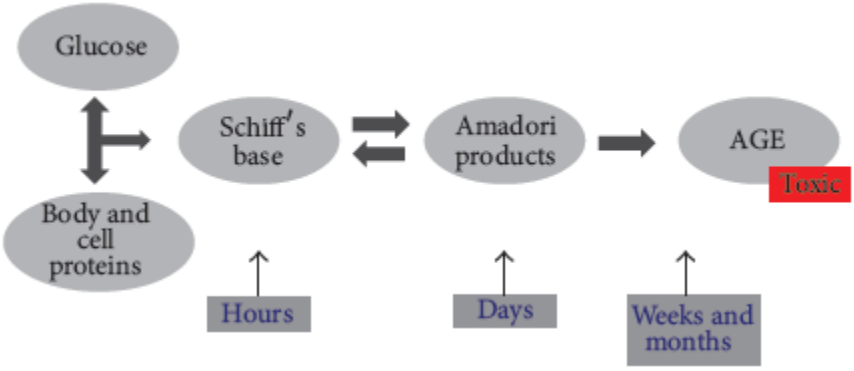


Fig 10: Formation of advanced glycation end products (AGEs).

(From Joanna M et al Review article)

OXIDATIVE STRESS

Oxidative stress may be defined as an imbalance between the level of ROS or oxygen radicals and the antioxidant defences in a biological system[42]. Oxidative stress induced by hyperglycaemia is an important pathway of diabetic microvascular complications [103], and increasing evidence suggests that the correlation between hyperglycaemia, changes in the redox homeostasis, and oxidative stress is the key event in the pathogenesis of diabetic retinopathy[43]. It has been hypothesised that both the development and the progression of retinopathy result from increased oxidative species[44].

RETINAL NEURODEGENERATION

It is now widely accepted that, in addition to the vascular changes, structural and functional damage to nonvascular cells (ganglion cells, glial cells, microglial) contributes to the pathogenesis of diabetic retinopathy[45]. There is evidence suggesting that neurodegeneration of retinal neurons and glial cells occurs even before the development of microaneurysms [46].

Dyslipidemia and Diabetic Retinopathy[6]

Hyperglycemia cause cell damage through the following pathways: the polyol pathway, overactivity of the hexosamine pathway, advanced glycation end product (AGE) formation with increased expression of AGE receptors, and activation of protein kinase C (PKC) isoforms[47]. The hyperglycemia-associated pathways PKC and AGE pathways interact with lipid levels.

Protein kinase C (PKC) is a family of 10 enzymes, in which the 1/2 isoform appears to be closely associated with the development of DR[31]. The expression of the PKC 1/2 isoform is enhanced in patients with diabetes. PKC upregulation contributes to the pathogenesis of DR in the form of differential synthesis of ECM proteins and ECM remodelling, enhanced release of angiogenic factors, endothelial and leukocyte dysfunction leading to capillary occlusion and leukostasis, and changes in blood flow to the retina[48].

AGEs are generated from nonenzymatic reactions between reducing sugars and lipoproteins[49]. In a highly oxidative environment such as retina, the accumulation of lipid and modification of protein will cause an accumulation of lipoxidation end products (ALEs).

There are two kinds of AGEs associated with DR pathogenesis: carboxyethylpyrrole [36] and malondialdehyde (MDA) [50]. AGEs are important pathogenic mediators of almost all diabetic complications. The interaction of AGEs with specific cell surface receptors has been implicated in the development of DR. These AGE receptors include the RAGE, galectin-3, CD36, and the macrophage scavenger receptor[51].

ApoA1, which is the structural protein of HDL-C, can promote vasoprotective mechanisms via its ability to promote reverse cholesterol transport from peripheral tissue to the liver and to inhibit LDL-C from oxidation, which may induce smooth muscle cell cytotoxicity and vascular endothelial dysfunction[52].

In the retina, ApoA1 is proposed as a key factor for preventing lipid accumulation [60] and a potent scavenger of oxygen-reactive species for protecting the retina from the oxidative stress caused by diabetes[53].

ApoB is the main component of LDL-C and is a reflection of atherogenicity[54]. Low ApoA1/ApoB ratio in serum is considered to be a risk for atherosclerosis[55]. Therefore, ApoB/A1 levels may reflect both damaging and protective lipoprotein pathways[56].

Treatment of Diabetic Retinopathy

Medical management of DR

1. Good glycemic control
2. Life style modification including weight reduction, increased level of exercise, smoking cessation
3. Good control of dyslipidaemia, hypertension, and BMI

Treatment for DME include ocular pharmacologic management and laser photocoagulation.

Ocular Pharmacologic management

1. Corticosteroids
2. Anti VGEF Drugs like ranibizumab, bevacizumab and pegaptanib.

Treatment for PDR

1. Anti VGEF drugs like bevacizumab
2. Surgical management by thermal laser photocoagulation and in sever condition panretinal photocoagulation.

DIABETIC NEPHROPATHY

Diabetic Nephropathy is the leading cause of end stage renal disease worldwide[11]. Patients with diabetes currently account for 35% of all the patients in ESRD being treated in USA and 28 % of ESRD patients in India[57]. Sixty three percent of patients with diabetic nephropathy have type-2 diabetes.

The development of diabetic nephropathy has a dramatic increase on the morbidity and mortality of patients with diabetes[57]. Diabetic Nephropathy rarely develops before ten years duration of diabetes[57]. The annual incidence of diabetic nephropathy in IDDM peaks just before 20 years and thereafter declines. Studies in type-2 diabetes show similar results to those in IDDM[57].

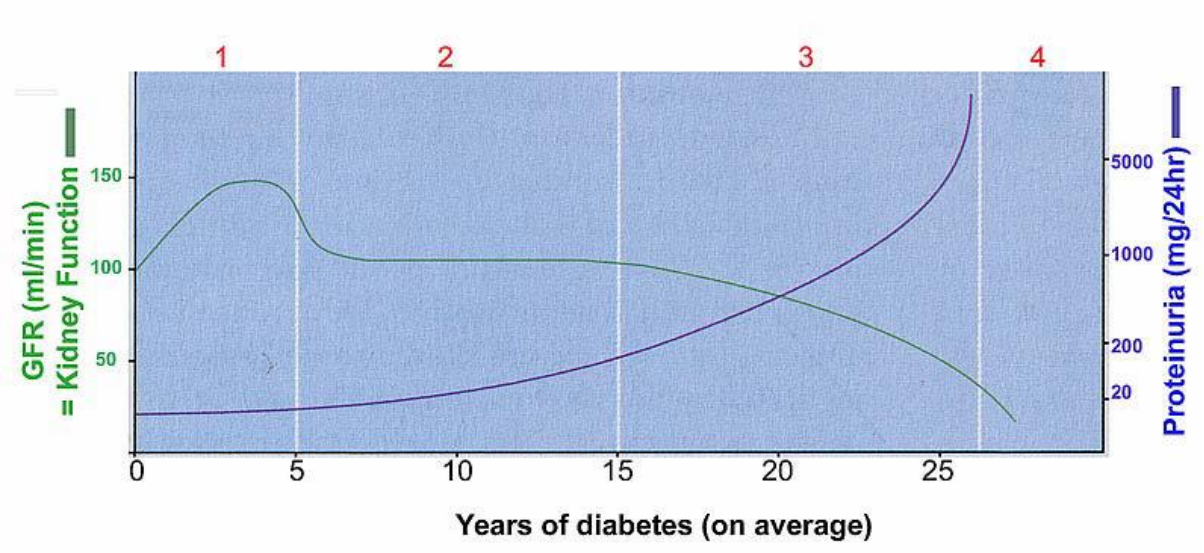


Fig 11: Natural history of Diabetic Nephropathy.

Natural history: [57]

Mogenson has classified Diabetic nephropathy into 5 stages.

Stage 1 (0-2 Yrs) - Stage of hyper filtration and hyper perfusion, is characterized by increased GFR, increase in renal size and reversible albuminuria unmasked by stress or exercise.

Stage 2 (2-5 Yrs.)- Stage of structural changes - Characterized by increase in glomerular basement membrane thickness and increase in mesangial matrix.

Stage 3 (5-10 Yrs.) - Stage of Microalbuminuria or incipient diabetic nephropathy -

Defined as a urinary albumin excretion rate of 20-200 μg/min or 30-300mg/24 hrs. Microalbuminuria is predictive of development of overt proteinuria.

Stage 4 (10-15 Yrs.)- Stage of overt proteinuria & renal failure - once proteinuria is established, renal function declines in exorably.

Stage 5 (15-25 Yrs.)- End stage renal disease - When GFR declines to less than 10 ml/min, the need for renal replacement in form of dialysis or renal transplantation is required.

Microalbuminuria

Microalbuminuria is defined as a urinary albumin excretion rate (AER) between 30-300mg/24hrs or 20-200μg/min in a timed specimen or 30-300mg/g of creatinine in a random specimen. Microalbuminuria develops in 30-40% of both type-1 and type-2 diabetes after duration of 20yrs[58].

The term Microalbuminuria is coined by Veberti et al to define such sub clinical elevation of Urine Albumin Excretion[59]. Microalbuminuria, a predictor for overt nephropathy and early cardiovascular mortality, is always associated with hypertension, hyperglycaemia, and

dyslipidemia. The importance of Microalbuminuria was first appreciated in the early 1980s when two landmark studies[60, 61] in London and Denmark independently reported that it was predictive of development of overt diabetic nephropathy and progressive renal failure[62]

Subsequently it was established that MA is related to cardiovascular mortality in diabetic population[63.64.65]. Later it was shown that MA among patients with diabetes reflects systemic vascular damage and increased risk of coronary heart disease independently of renal function[66].

The ADA /ACC then in 1998 included positive microalbuminuria /macro albuminuria test as risk factor of CAD in diabetic subjects[67]. Exercise within 24h, infection, fever, congestive heart failure, marked hyperglycaemia, marked hypertension, pyuria, haematuria and contamination of the specimen with vaginal fluid may elevate urinary albumin excretion over baseline values.

Methods[68]

1) Dipstick method

2) Semi quantitative method

• Chemical precipitation (sulphosalicylic acid trichloroacetic acid)

• Immunoprecipitation (Micral test)

3) Turbidity methods

• Photometric (Chrmacyn blue method)

• Nephelometry

4) Sensitive quantitative methods

• Radio immunoassay

• Cellulose acetate, agarose gel electrophoresis

Measurement of albumin excretion in a 24-h urine collection has long been the" gold standard "for quantitative evaluation of albuminuria in diabetic patients[69].

For the convenience and consistency, the American Diabetic Association and the National Kidney Foundation[70] have recently recommended measurement of albumin to creatinine ratio (ACR) in a random spot urine collection for diagnosis of microalbuminuria.

The guidelines recommended using a first-morning sample because of the potentially higher correlation with 24-h albumin excretion, but a random sample is considered acceptable if a first-morning specimen is not available. Overnight samples can be used to distinguish true micro albuminuria from postural or exercised proteinuria, which is common in young patients.

Pathogenesis and Pathophysiology:

The earliest morphologic abnormality in diabetic nephropathy is the thickening of the glomerular basement membrane (GBM) and expansion of the mesangium due to accumulation of extra cellular matrix.

Light microscopy findings show an increase in the solid spaces of the tuft, most frequently observed as coarse branching of solid (positive periodic-acid Schiff reaction) material (diffuse diabetic glomerulopathy). Large acellular accumulations also may be observed

within these areas. These are circular on section and are known as the Kimmelstiel-Wilson lesions/nodules.

Electron microscopy provides a more detailed definition of the structures involved. In advanced disease, the mesangial regions occupy a large proportion of the tuft, with prominent matrix content. Further, the basement membrane in the capillary walls is thicker than normal.

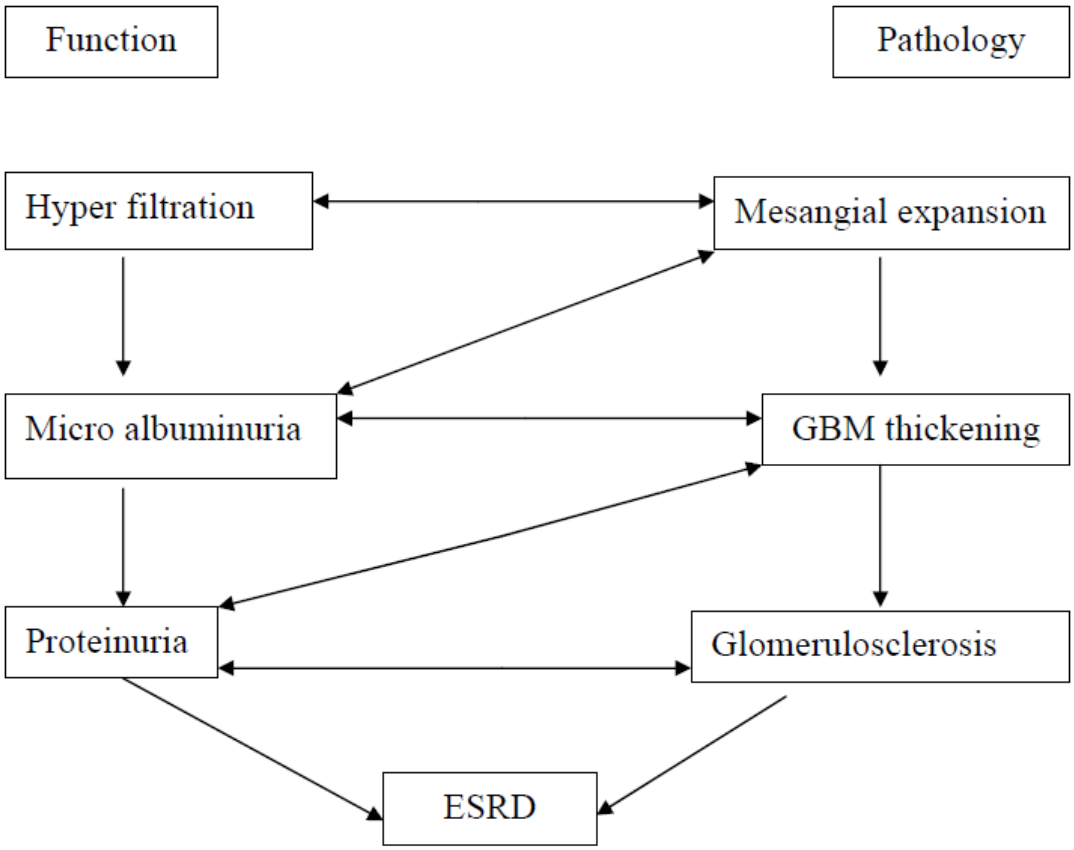


Fig 12: Correlation between function and pathology in Diabetic Nephropathy[58].

The severity of diabetic glomerulopathy is estimated by the thickness of the peripheral basement membrane and mesangium and matrix expressed as a fraction of appropriate spaces (e.g., volume fraction of mesangium/glomerulus, matrix/mesangium, or matrix/glomerulus).

Three major histological changes occur in the glomeruli of persons with diabetic nephropathy.

First, mesangial expansion is directly induced by hyperglycaemia, perhaps via increased matrix production or glycosylation of matrix proteins.

Second, GBM thickening occurs.

Third, glomerular sclerosis is caused by intraglomerular hypertension (induced by renal vasodilatation or from ischemic injury induced by hyaline narrowing of the vessels supplying the glomeruli). These different histological patterns appear to have similar prognostic significance.

Hyperglycemia increases the expression of transforming growth factor-beta (TGF-beta) in the glomeruli and of matrix proteins specifically stimulated by this cytokine. TGF-beta may contribute to both the cellular hypertrophy and enhanced collagen synthesis observed in persons with diabetic nephropathy.

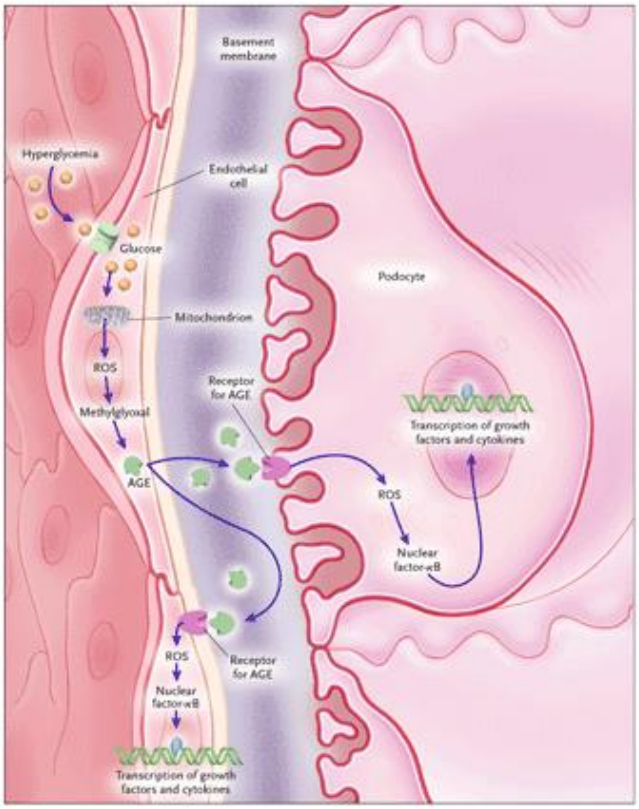


Fig 13: Endothelial-Cell Damage in Hyperglycemia[71].

Hyperglycemia drives more glucose into endothelial cells, causing excessive generation of reactive oxygen species (ROS) in mitochondria; these, in turn, favor the generation of methylglyoxal and advanced glycation end products (AGE).

These end products are bound by specific receptors, causing a burst of reactive oxygen species and activation of the transcription factor nuclear factor- B and, ultimately, transcription of injurious growth factors and cytokines on endothelial cells as well as neighbouring cells, such as those within the kidney, mesangial cells, and podocytes.

Hyperglycemia also may activate protein kinase C, which may contribute to renal disease and other vascular complications of diabetes.

Genetic factors: [57]

A family history of hypertension is also associated with increased risk of diabetic nephropathy.

An association between RBC sodium-lithium counter transport activity, viewed as a marker of the risk for essential hypertension and development of diabetic nephropathy is supported by Meta - analysis of various studies.

Polymorphism of genes such as ACE and angiotensin type I receptor have been assessed. ACE gene polymorphism may represent a genetic determinant of renal response of an individual to inhibition of ACE.

Hemodynamic Factors: [57]

Diabetic Nephropathy is commonly associated with systemic hypertension.

Longitudinal studies in patients with IDDM have suggested that in the transition from Normoalbuminuria to microalbuminuria, there is a modest rise in blood pressure of about 3 mm Hg per year best detected by ambulatory blood pressure monitoring.

Micro puncture studies in animal models of diabetes show raised intra glomerular pressure. These renal hemodynamic changes may be partly due to the actions of vasoactive hormones such as angiotensin II and endothelin.

ACE inhibitors and angiotensin II receptor antagonists; reduce elevated intra glomerular pressure and thus the beneficial effects of ACE inhibitors in diabetic nephropathy. The

hallmark of pathological changes in diabetic nephropathy is increased extra cellular matrix accumulation.

It has been postulated that the cytokine – Transforming Growth Factor - B (TGF - B) which is stimulated by glucose, AGE, Vasoactive, hormones such as angiotensin II and endothelium, plays a pivotal role in the development of Diabetic Nephropathy.

Diabetic Nephropathy and Dyslipidaemia

Besides hypertension, glycemic control and genetic predispose to diabetic dyslipidaemia and plays an important role in the pathophysiology and progression of vascular disease and probably diabetic nephropathy as well[72]. Diabetic Nephropathy per se, in addition to diabetes, impairs lipid metabolism[73] mainly, but not exclusively, via urinary protein execration.

Clinical significance of dyslipidemia in type- 2 diabetic nephropathy

Elevated LDL cholesterol is a major risk factor for CHD[74]. Some elevation of LDL cholesterol appears to be necessary for the initiation and progression of atherosclerosis.

The development of nephropathy accelerates vascular damage and is strongly related to early cardiovascular morbidity and mortality in type- 1 and type- 2 diabetic patients[75].

The Multiple Risk Factor Intervention Trial (MRFIT) has demonstrated that high serum cholesterol confers a two-four fold increased risk in diabetic patients for developing cardiovascular events than in non-diabetic subjects[76]. Uusitupa et al[77] were able to demonstrate that, serum total triglycerides, the compositional abnormalities of lipoproteins,

evidently associated with disturbed catabolism of VLDL, were related to cardiovascular mortality.

Role of Lp (a)

Lp (a) is an independent risk factor for the progression of diabetic nephropathy in type- 2 diabetic patients with overt proteinuria.

Patients with nephropathy had significantly higher Lp (a) levels than patients without nephropathy[78]. Also, serum Lp (a) concentrations were increased significantly with increasing urinary albumin excretion.

In patients with proteinuria (microalbuminuria and albuminuria), high Lp (a) levels are probably because of decreased renal excretion, rather than raised Lp (a) causing nephropathy. Dyslipidemia and the progression of diabetic nephropathy

In experimental animals, hyperlipidemia has been implicated in causing renal injury. It has been shown that altered serum lipoproteins interact with structures of the glomerules. LDL particles modified by glycosylation and oxidation exhibits enhanced binding to glycosaminoglycans of the glomerular basement membrane. In addition, the deposition of modified altered LDL particles in the mesangium may induce chemotactic signals for macrophages and stimulate mesangial cell proliferation[79].

The preferential scavenger receptor mediated uptake of modified LDL by monocytes/macrophages has recently been considered to cause the formation of glomerular

and mesangial foam cells[80]. Other mechanisms include mesangial expansion by accumulation of apoB and apoE leading to a reduction in glomerular filtration surface area, an alteration in renal cortical tissue lipids, alterations in membrane fluidity and function induced by disturbances in fatty acid concentrations and alterations in glomerular hemodynamics[82].

In diabetic nephropathy, hyperlipidemia has been identified as a risk factor for a more rapid rate of decline in GFR and increased mortality[81].

Especially, an increased plasma concentration of triglycerides-rich apoB-containing lipoproteins was found to be linked to a more rapid decrease in renal function and the combination of dyslipoproteinaemia and hypertension appears to act synergistically to indicate a more rapid decline of renal function.

Recently, high triglycerides and low HDL cholesterol could be identified as strong predictors of more rapid progression of microalbuminuria in type- 2 diabetic patients with well-controlled blood pressure[79].

Management of diabetic nephropathy

Strategies for microalbuminuria

1) Meticulous Glycemic and Blood pressure control.

2) Avoid dehydration.

3) Avoid urinary tract infection.

4) Use of ACE inhibitors (ACEIs) in normotensive patients.

Strategies in clinical nephropathy

1) Meticulous Glycemic and BP control.
2) Cessation of smoking.
3) Salt restriction.
4) Protein restriction (0.4.0.6mg/kg/day).
5) Treat associated lipid disorders.
6) Check for urinary tract infection.
7) Avoid dehydration.

Caution against use of drugs which harm renal function and radiographic dyes; this should always be done in any diabetic, but all the more in patients with clinical nephropathy.

End Stage Renal Disease -Renal replacement therapy (dialysis and / or renal transplant) is the treatment for end stage renal disease (ESRD).

DIABETIC NEUROPATHY

Definition

According to San Antonio conference[83] defined as "Diabetic neuropathy is a descriptive term meaning a demonstrable disorder, either clinically evident or subclinical, that occurs in the setting of diabetes mellitus without other causes of peripheral neuropathy, including manifestations in somatic and/or autonomic parts of the peripheral nervous system".

Epidemiology

Dyck and colleagues[84] studied diabetes in Minnesota, found that 54% with type 1 diabetes and 45% with type 2 diabetes had poly neuropathy.

Neuropathy is most common in diabetics older than 50 years and is infrequent in those younger than 30 years and rare in childhood.

The prevalence of diabetic neuropathy from one of the study done in urban south India population in 2008 was 26.1%[85].

In other study done in north India in 2015 showed prevalence of diabetic neuropathy was 60.7%.

Classification

The first classification was suggested by Leyden[86] and Pryce in 1893 into three forms of neuropathy.

Hyperesthetic

Paralytic

Ataxic

Most widely used classification was proposed by Thomas[87] in 1973.

Clinical classification of diabetic neuropathies

- Symmetric neuropathies :

Diabetic polyneuropathy

Painful autonomic neuropathy

Painful distal neuropathy with weight loss “diabetic cachexia”

Insulin neuritis

Polyneuropathy after ketoacidosis

Polyneuropathy with glucose impairment

Chronic inflammatory demyelinating polyneuropathy with diabetes mellitus

- Asymmetric neuropathies :

    Cranial neuropathy

    Mononeuropathies

Median neuropathy at wrist

Ulnar neuropathy at elbow

Peroneal neuropathy at the fibular head

Radiculoplexoneuropathies:

    Lumbosacral

    Thoracic

    Cervical

Pathogenesis

The pathogenesis of diabetic polyneuropathy is multifactorial with genetic, environmental, behavioural, metabolic, neurotrophic and vascular factors[88]. These pathogenic factors act synergistically. Many of these hypothesis are based on animal models of diabetes, but none of these truly reproduce the changes as seen in diabetic neuropathies in humans.

The possible mechanisms are

1. Hyperglycaemia and polyol pathway
2. Advanced glycation end products(AGE)
3. Free radical and oxidative stress
4. Biochemical abnormalities
5. Vascular and haematological abnormalities
6. Defects in nerve regeneration

Long-term hyperglycemia causes downstream metabolic cascades of polyol pathway hyperactivity, advanced glycation end-products (AGE)/receptor for AGE (RAGE) reactions and increased reactive oxygen species (ROS).

They compromise both endoneurial microvessels and neural tissues themselves through activation of poly-ADP-ribose polymerase (PARP), alterations of protein kinase C (PKC) and an increase in mitogen-activated protein kinase (MAPK), as well as activation of nuclear factor-(NF)-kB, resulting in functional and structural changes of peripheral neuropathy.

Metabolic aberrations in the nerve elicit pro-inflammatory reactions, inducing release of cytokines, suppression of neurotrophins and migration of macrophages, and promote the development of neuropathy.

Recently, cellular factors derived from the bone marrow were found to produce chimeric cells in peripheral nerves of diabetic animals to elicit nerve injury.

There is also the possibility that other cellular components from the bone marrow have an influence on the nerve pathology in diabetes.

In addition, ischemia/reperfusion might also accelerate nerve injury, in part mediated by inflammatory reactions.

Risk factors represented by hypertension, hyperlipidemia, smoking and insulin resistance are also important contributors to the development of neuropathy[89].

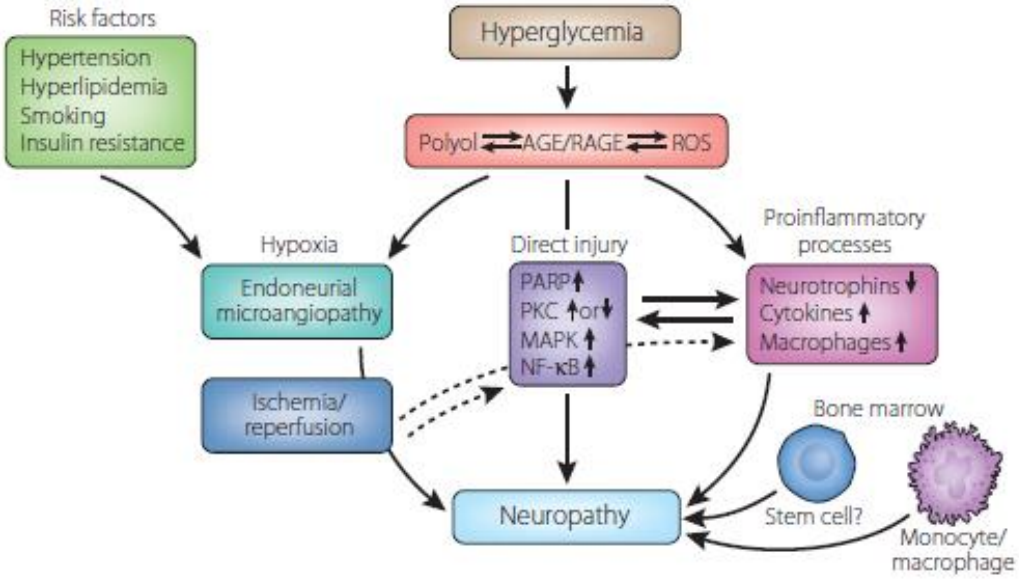


Fig 14: Summary of pathogenetic mechanisms of diabetic neuropathy.

(From review article by Yagihashi S et al[89])

Staging of Diabetic Neuropathy

The following criteria for staged severity suggested by Dyck[90] are generally accepted and recommended:

Stage 0: no neuropathy. No symptoms and less than two abnormalities on testing (neurologic examination, NCS, quantitative sensory testing and autonomic function tests)

Stage 1: Asymptomatic neuropathy

NIa: no symptoms but two or more abnormal tests

Nib: NIa + weakness in foot dorsiflexion

Stage 2: symptomatic neuropathy

N2a: without weakness of foot dorsiflexion

N2b: N2a + weakness of foot dorsiflexion

Stage 3: Disabling neuropathy: Disabling motor, sensory. Autonomic features and two or more abnormal tests.

DYSLIPIDEMIA IN DIABETIC NEUROPATHY

Dyslipidemia contributing to the development of diabetic neuropathy may explain the earlier incidence of diabetic neuropathy in individuals with type 2 diabetes mellitus.

Lipid profiles are commonly abnormal early in the course of type 2 diabetes in a temporal pattern that correlates with the presence of diabetic neuropathy. Data from several large scale trials of patients with type 2 diabetes tells early dyslipidemia as a major independent risk factor for the development of diabetic neuropathy[91].

In the United Kingdom Prospective Diabetes Study (UKPDS), 3,867 newly diagnosed type 2 patients there was no difference in the development of diabetic neuropathy between the two groups, which had similar lipid and blood pressure profiles (1998).

Further molecular and genetic studies are needed to establish the exact mechanism how dyslipidemia causes diabetic neuropathy.

Treatment of diabetic neuropathy

1. Therapies to modify the course of Diabetic neuropathy
2. Symptomatic treatment of Diabetic Neuropathy

Therapies to modify the course of Diabetic neuropathy

1. Optimized glycemic control
2. Measures that may become clinically useful
    a. Aldose reductase inhibitors
    b. Essential free fatty acids

c. Vasodilator drugs like alpha adrenergic antagonists, calcium channel blocking agents, angiotensin converting enzyme inhibitors, various prostonoids and nitrates

3. Strategies under experimental investigation
    a. Inhibition of glycation like aminoguanidine
    b. Antioxidants like alpha lipoic acid
    c. Agents that promote nerve growth and repair like nerve growth factor and gangliosides

Symptomatic treatment of Diabetic Neuropathy

1. Capsaicin – superficial hyperasthesia with burning and dysasethetic pain
2. Tricyclic antidepressants effective in controlling burning pain like imipramine, amitriptyline and desipramine.
3. Anticonvulsants like carbamazepine, gabapentin, phenytoin, valproate, clonazepam effective in lancinating pain
4. Others like baclofen, clonidine, lignocaine and tramadol hydrochloride

Non pharmacologic therapies that have also been tried with limited success are sympathectomy, spinal cord blockade, and electrical spinal cord stimulation.

DYSLIPIDEMIA IN TYPE- 2 DIABETES

Lipoproteins are spherical particles made up of hundreds of lipid and protein molecules. They are smaller than red blood cells. The major lipids of lipoproteins are cholesterol, triglycerides and phospholipids.

Triglycerides and cholesterol esters are non-polar[92] lipids that are insoluble in aqueous environments and comprise the core of lipoproteins**.**

Phospholipids and free cholesterol, which are soluble in both lipid and aqueous environments, cover the surface of the particles where they act as the interface between the plasma and core components.

The apolipoproteins, a family of proteins also occupies the surface of the lipoproteins and plays crucial role in the regulation of lipid transportation and lipoprotein metabolism.

Bloor's[93] classification of lipids

1. **Simple lipids**:

They are esters of fatty acids with various alcohols:

a. Neutral Fat: triglycerides

b. Waxes: True waxes, cholesterol esters, Vitamin A, Vitamin D.

2. **Compound lipids**

Esters of fatty acids containing groups in addition to an alcohol and a fatty acid.

a. Phospholipids

b. Glycolipids (glycosphingolipids)

c. Sulpholipids

d. Aminolipids

e. Lipoproteins

3. **Derived Lipids**

a. Fatty acids

b. Monoglycerol

c. Alcohols

Lipids are carried in plasma in the form of lipoprotein complexes. These complexes impart solubility to the otherwise insoluble lipids.

Cellular lipids

The cellular lipids are:

1. Structural lipids: An inherent part of the membranes and other parts of cells.
2. Neutral fat: Stored in the adipose cells of the fat depots.

Neutral fat is mobilized during starvation, but structural lipid is preserved. The fat depots vary in size. In non-obese individuals, they make up about 15% of body weight in men and 21% in women.

In the depots, glucose is metabolized to fatty acids and neutral fats are synthesized. Neutral fat is also broken down, and free fatty acids are released into the circulation.

A special type of lipid called brown fat makes up a small percentage of total body fat. It is located between the scapulas, at the nape of the neck, along the great vessels in the thorax and abdomen, and in other scattered locations in the body. In brown fat depots, the fat cells as well as the blood vessels have extensive sympathetic innervations.39

Cholesterol:

Cholesterol is the best-known steroid because of its association with atherosclerosis. It is also significant because it is the precursor of a large number of equally important steroids, which include the bile acids, adrenocortical hormones, sex hormones and cardiac glycosides.

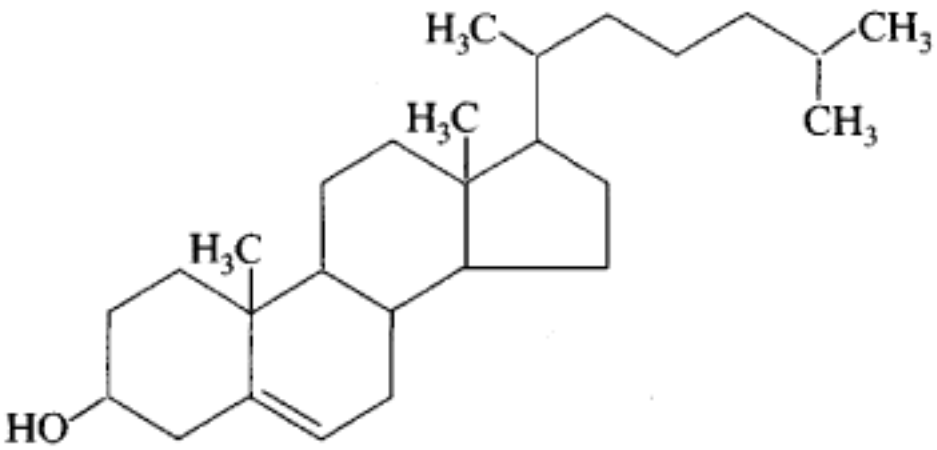


Fig 15: Molecular Structure of Cholesterol.

The structure consists of a cyclic nucleus resembling phenanthrene to which a cyclopentane ring is attached.

The hexagonal ring denotes a completely saturated six-carbon ring with all valences satisfied by hydrogen bonds. Methyl side chains are shown as single bonds unattached at the farther end.

Cholesterol is widely distributed in all cells of the body, but particularly in nervous tissue. It is a major constituent of the plasma membrane and of plasma lipoproteins.

Peroxidation (auto-oxidation) of lipids exposed to oxygen is responsible for damage to tissues in vivo, where it may be a cause of, inflammatory disease, atherosclerosis, cancer, ageing etc.

The deleterious effects are caused by free radicals (ROO1, RO', OH1) produced during peroxide formation from fatty acids containing methylene-interrupted double bonds.

Biosynthesis:

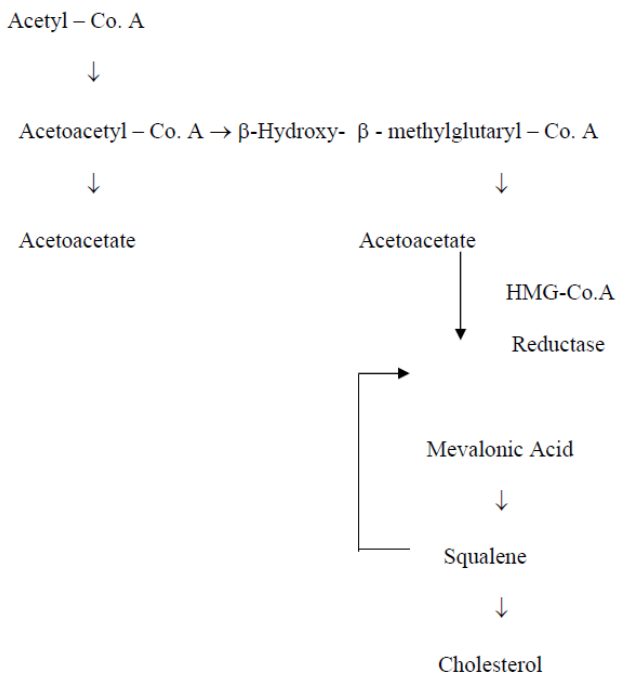


Fig 16: Biosynthesis of Cholesterol.

Lipoproteins

Lipoproteins are combinations of fat and protein. They are macromolecular complexes that carry hydrophobic lipids, particularly cholesterol and triglyceride in the plasma and are important cellular constituents, occurring both in the cell membrane and in the mitochondria. The major lipids of lipoproteins are cholesterol, triglycerides and phospholipids.

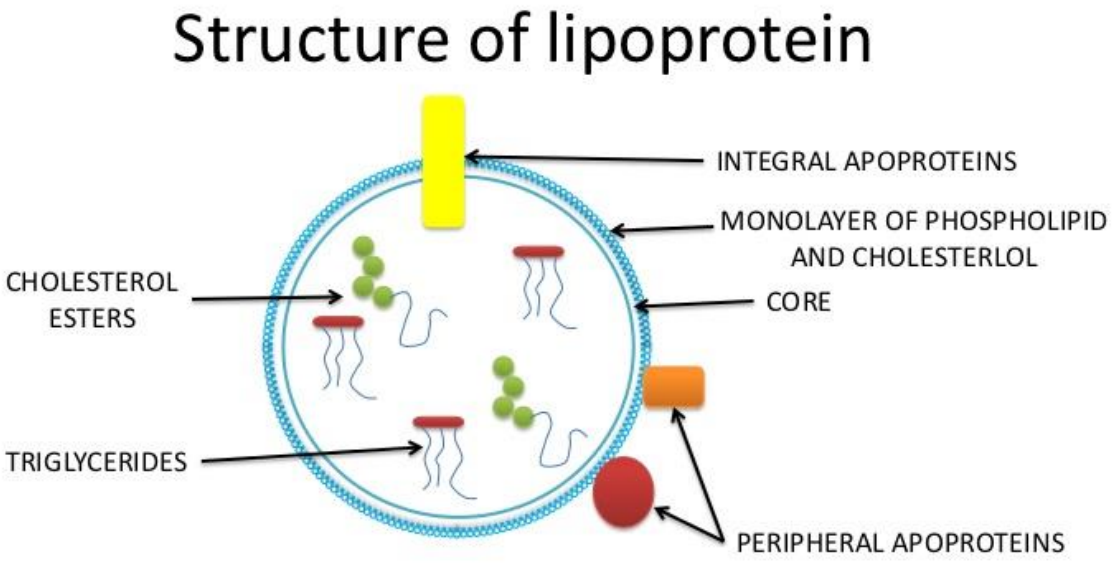


Fig 17: Structure of Lipoprotein.

Courtesy: Slideshare.net

Lipoproteins of Plasma[95]

The major plasma lipids including cholesterol and triglyceride do not circulate freely41 in solution in blood but rather are transported in the form of lipoprotein complex.

These complexes of lipids and protein impart solubility to the otherwise insoluble lipids and all lipids enter and travel through the blood stream as lipoprotein complexes.

These various types of lipoproteins can be separated by precipitation, electrophoresis of differential centrifugation using methods like those used in ordinary proteins.

The interaction of lipoproteins with cell membrane receptors and thus their metabolic fate is also dictated largely by the nature of protein moieties.

Apolipoproteins

Apolipoproteins provide structural stability to the lipoprotein[96], play important role in lipoprotein formation and metabolism including enzyme activation or inhibition and act as ligands for lipoprotein receptors.

VLDL, except after a fat containing meal, account for most of the triglycerides in the plasma. They vary in size (30 to 80 nm), mainly because of different triglyceride content (20 to 80 % by weight).

LDL (about 20 nm) carries most (about two-thirds) of the cholesterol in normal plasma. The LDL is about 50 % by weight cholesterol and 20 % by weight protein. HDL molecules (about 8 nm) are about 50 % of proteins by mass and 50% of lipid hence their high density.

The relatively high content of triglyceride in chylomicrons and VLDL is a reflection of their principal roles in the transport of triglyceride from the intestine to the liver and from the liver to the other tissues respectively.

There is evidence indicating the LDL is formed from the VLDL fraction by the removal of triglycerides (Gitten D et al., 1958).

Table 1: Major lipoprotein classes

Lipoprotein	Density g/ml	Size in mm	Apolipoproteins		Other constituents
			Major	Other	
chylomicrons	0.930	75-1200	Apo B – 48	A-I, IV C-I,II,III	Retinyl esters
Chylomicron remnant	0.930-1.006	30.80	Apo B - 48	E, A-1,IV, C-I,II,III	Retinyl esters
VLDL	0.930-1.006	30.80	Apo B - 100	A-1,II,V C-I,II,III	Vit E
IDL	1.006-1.019	23.35	Apo B	E, C-1,II,III	Vit E
LDL	1.019-1.063	18.25	Apo B	-	Vit E
HDL	1.063-1.210	5.12	Apo A1	A-II,IV E,C-III	LCAT,CETP Paroxanose
Lp(a)	1.050-1.120	25	Apo B-100	Apo(a)	-

LCAT – Lecithin cholesterol acyl transferase

CETP- Cholesteryl ester transfer protein

Lipoprotein Metabolism[97]

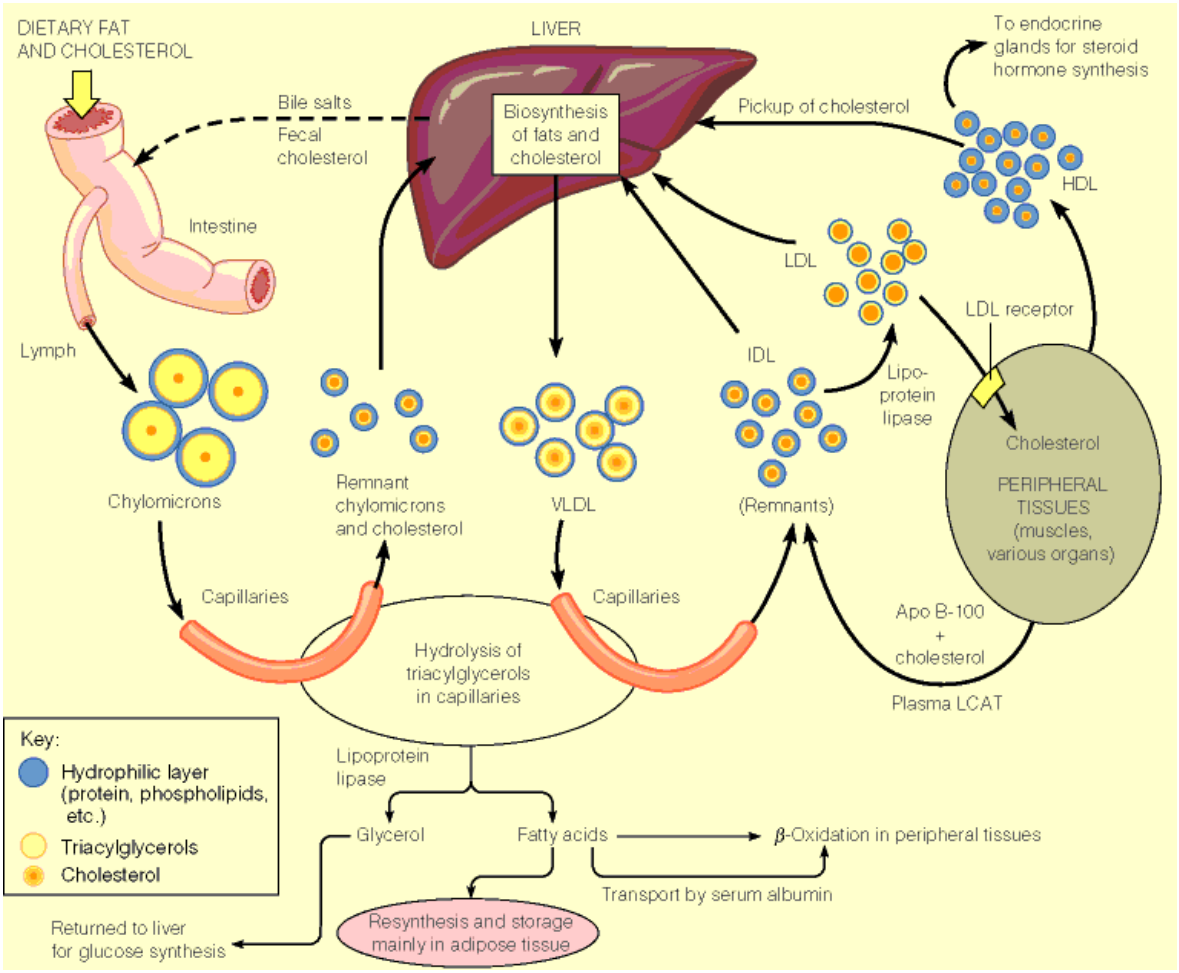


Fig 18: Lipid metabolism pathway.

Courtesy: APSU Biology

Chylomicrons

These are normally found in plasma only after meal and transport dietary fat from the small intestine to the blood stream via the lymphatic and thoracic duct. These large triglyceride-rich particles bind to the capillary endothelium of skeletal muscle and adipose tissue hence their triglyceride content is hydrolyzed by lipoprotein lipase to nonesterified fatty acids.

These are either oxidized for fuel or taken up and re-esterified in adipose tissue for storage as triglyceride. After losing its triglyceride the chylomicrons remnant particle is taken up by the liver via a receptor-mediated process involving apoprotein E.

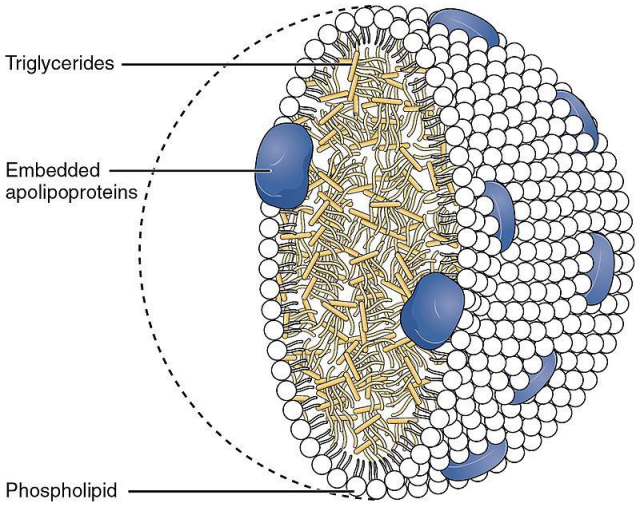


Fig 19: Chylomicron structure.

Courtesy: en.wikipedia.com

ApoA, ApoB, ApoC, ApoE (apolipoproteins); T (triacylglycerol); C (cholesterol); green (phospholipids)

VLDL

These are formed in the liver and to a lesser extent in the intestine. They transport endogenously synthesized triglyceride and, as with chylomicrons, their triglyceride is hydrolyzed by lipoprotein lipase.

During VLDL catabolism some surface components such as phospholipids and apo C transfer to HDL particles but most of the apoprotein B and cholesterol is conserved. The final

products of VLDL catabolism are VLDL remnant particles or IDL, which can either be taken up directly by the liver or converted to LDL.

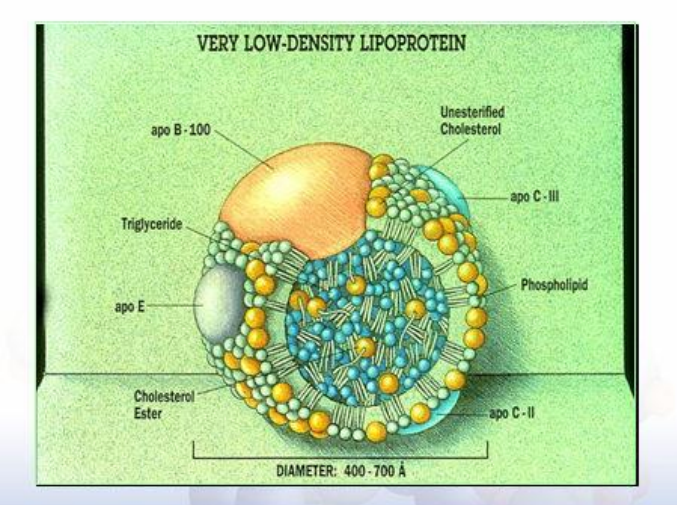


Fig 20: Structure of VLDL.

Courtesy: Slideshare.net

LDL

These deliver cholesterol to extra-hepatic tissues and account for approximately 70 % of total plasma cholesterol. LDL binds to specific high affinity receptors on the cell surface, which recognize and bind LDL apoprotein B. Receptor bound LDL is then internalized by a process called absorptive endocytosis.

The resulting vesicle fuses with cellular lysozymes in which the LDL cholesterol ester is hydrolyzed to free cholesterol, which diffuses to the cell cytoplasm where it may be used for membrane or steroid hormone synthesis. Cellular free cholesterol also regulates cholesterol metabolism by suppressing cholesterol synthesis, stimulating its own reesterification and suppressing synthesis of LDL receptors. This receptor-mediated pathway removes one-to two-thirds of plasma LDL.

A less well understood mechanism for the removal of LDL is the "scavenger pathway" which may lead to accumulation of cholesterol esters in scavenger cells of the monocyte-macrophage system.

The scavenger pathway98, 99 is mediated through acetyl-LDL receptors or scavenger receptors; these receptors are characterized by their ability to interact with chemically modified LDL but not with native LDL. LDL particles that have been modified by acetylation, acetoacetylation or reaction with malondialdehyde were taken up by high affinity cell surface receptors on macrophage resulting in marked cholesterol accumulation.

Furthermore, macrophage can also alter LDL[100] so that these particles can be taken up by macrophages in an unregulated manner. The physiologically important LDL modification probably involves oxidation and results in lipid peroxidation.

The oxidized LDL may directly take part in atherogenesis because they are cytotoxic, may serve as chemo attractants for circulating monocyte –macrophages, and are immunogenic.

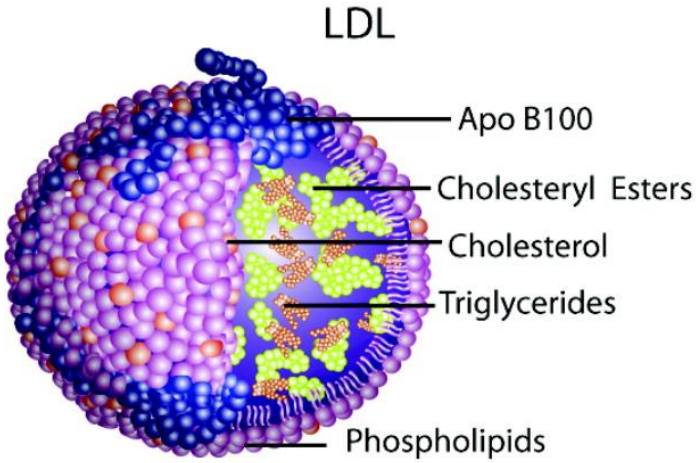


Fig 21: Structure of LDL.

Courtesy: Scientificpsychic.com

HDL

These particles, which are synthesized directly by the liver and intestine or created as by-product of the metabolism of triglyceride-rich lipoproteins, account for 20 to 30 % of total plasma cholesterol.

There is evidence that HDL may be involved in reverse cholesterol transport, i.e. transport from the peripheral cells back to the liver.

Reverse cholesterol transport[101] from peripheral cells to the liver involves in the first step small particles of discoidal shape, pre ß-HDL, synthesized in liver and small intestine, or resulting from hydrolysis of triglyceride-rich particles.

These pre ß-HDL uptake cholesterol from peripheral cells, and their shape change to spherical particles, named HDL3 then HDL2, as they become enriched in esterified cholesterol (via an esterifying enzyme, lecithin cholesterol acyl transferase (LCAT) associated with pre ß-HDL particles) and phospholipids. The final uptake of HDL2 by the liver involves a selective receptor, named scavenger receptor B1 (SR-B1).

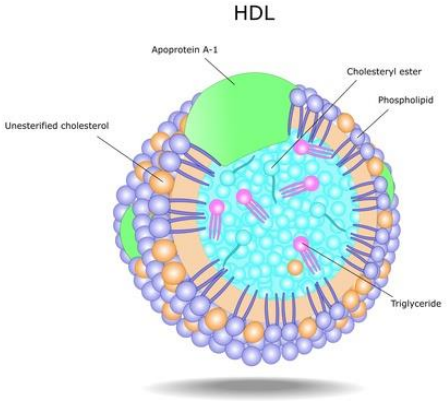


Fig 22: Structure of HDL.

Courtesy: slideshare.net

Lipoprotein (a) or Lp (a)

Kare Berg detected Lipoprotein (a) in 1963 in Norway[102]. Lp (a) was purified to homogeneity and characterized in some detail in early 1970's.

Lp (a) was rediscovered several times and designated "sinking pre β- lipoprotein", pre β-1 Lipoprotein, or just a "new" or atypical lipoprotein[103].

A breakthrough in Lp (a) research was the cloning and sequencing of Apo (a) by Mc Lean et al, which revealed a high degree of homology of Apo (a) with plasminogen.49

The clinical interest in Lp (a) arose when Dahlen and co-workers recognized a higher frequency of Lp (a) positive subjects among men with coronary artery disease as compared with the controls[104].

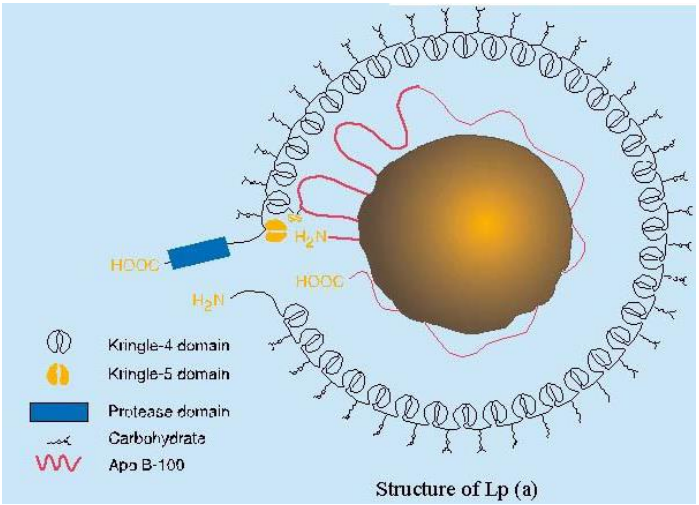


Fig 23: Structure of Lp (a)

Courtesy: Belgian Lipid Club

Lp (a) is a complex assembled[105] from two different components. One is an LDL and contains all the lipid with hydrophobic apo B-100; other is a hydrophilic glycoprotein apo (a). Both LDL and apo (a) are believed to be linked by a single disulphide bond.

Apo (a) has a strong structural homology to plasminogen, and their genes are adjacent on chromosome 6. Sequencing of apo (a) at the protein level has revealed a high degree of homology with plasminogen.

Plasminogen is a plasma serine protease of the fibrinolytic system. Although the normal function of Lp (a) is unknown the close homology between Lp (a) and plasminogen has raised the possibility that this lipoprotein may inhibit endogenous fibrinolysis by competing with plasminogen for binding on the endothelial surface.

Apo (a) may also induce monocyte chemotactic activity in the vascular endotheliumx. This mechanism may contribute to a role of Lp (a) in atherothrombosis.

Metabolism of Lp (a) [105,106,107]

Liver primarily secretes plasma apo (a). Apo (a) mRNA is present in significant amounts in liver, testis and brain but is most abundant in liver. Since the liver is the only organ that also produces Apo B, it is the only organ with potential to assemble Lp (a). Direct evidence for the role of the liver in Apo (a) production came from studies of patients undergoing therapeutic liver transplantation. Such patients may change their genetic apo (a) type completely following transplantation and acquire the phenotype of the liver donor. It is

unclear where Lp (a) is assembled. Assembly from LDL and apo (a) in plasma has been demonstrated in vitro and in plasma of mice transgenic for the human
apo (a) gene.

Plasma Lp (a) concentration are primarily determined by the rate of synthesis rather than by catabolism. The mechanism and sites of Lp (a) catabolism are unknown. The LDL- receptor pathway seems to play only a minor role if any.

Genetics of Lp (a)

The Apo (a) component of Lp (a) is a complex molecule composed in part varying number of cysteine repeats that results in great heterogeneity underlying its molecular complexity, more than 25 heritable isoforms of Lp (a) exists. This molecular variability has clinical importance because of Lp (a) levels vary widely across ethnic groups.

Lp (a) is a quantitative genetic trait. The distribution of Lp (a) in the population is highly skewed and very broad.

Average Lp (a) concentration is significantly different among population, with Asians having the lowest and Africans having the highest plasma Lp (a) levels.

Table 2: Characteristics of Lp(a) and Apo (a)[108,109]

Lp (a)	
Electrophoretic mobility (agarose)	Pre beta
Buoyant density (g/ml)	1.040 –1.131
Isoelectric point (pl)	4.9
Molecular mass (Daltons)	3.8-4.60 x 10^{-6}
Molecular diameter (A0)	4.9
Plasma concentration (mg/dl)	0.1 - 120
Protein (g/mol)	800000 - 1350000
Free cholesterol ester mol/mol	750
Cholesterol ester (mol/mol)	800000 - 1350000
Triglycerides (mol/mol)	750
Phospholipid (mol/mol)	2000
Fractional catabolic rate (per day)	1110
Plasma t1/2 (days)	3.32 – 3.93
Synthetic rate (mg/kg/day)	4.60 _+ 3.64 (0.54 – 11.39)
Apo (a)	
Molecular mass (Daltons)	300000-800000
Amino acids in mature protein	4529
Carbohydrate (%)	28
Sialic acid (%)	21

MAJOR APOLIPOPROTEINS AND THEIR ROLES IN METABOLISM

The apolipoprotein constituents of the major plasma lipoproteins can be visualized by sodium dodecyl sulphate- polyacrylamide gel electrophoresis.

It established that apolipoproteins of the various lipoproteins regulate lipoprotein metabolism and determine the unique roles of these lipoproteins in lipid metabolism.

Several major functions of lipids have been described to specific apolipoproteins. One established function is involvement in the transport and redistribution of lipids among various tissues. The delivery of lipids to specific cells involves the recognition of specific apolipoproteins by cell surface lipoprotein receptors. Apolipoproteins also acts as cofactors for enzymes in lipid metabolism.

The cofactors in some lipid metabolism activate LCAT by providing a suitable lipid or liposome interface on which the enzyme act. In addition specific apolipoproteins play their role in the maintenance of structure of lipoproteins. Various apolipoproteins, e.g., apoB, A-I, and E, stabilize the micellar structure of the lipoproteins and function, in association with phospho- lipids on the surface of the particles, to provide a hydrophilic surface.

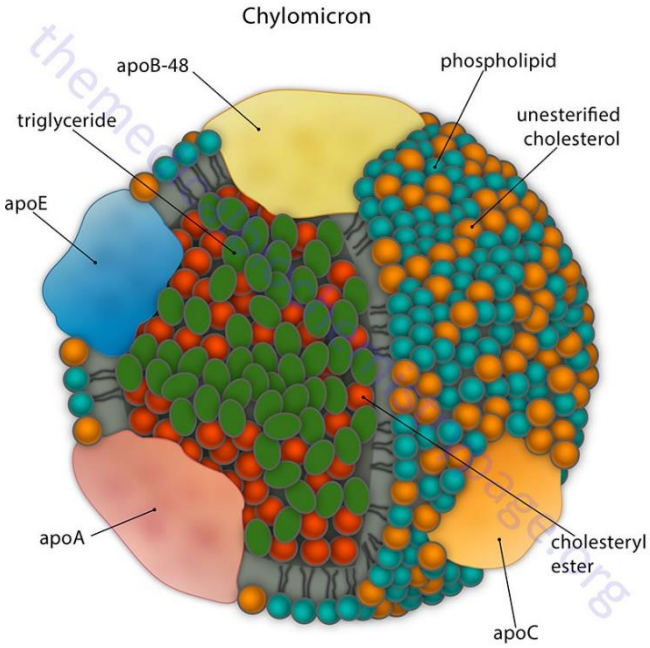


Fig 24: Structure of lipoprotein particle.

Courtesy: The medical biochemistry

Table 3: Classification of Apoproteins[95]

Apoprotein	(MW)	Function and Comments
ApoA-I	(29kDa)	Major protein of HDL, activates LCAT, high levels of apoAI are associated with a reduced risk of CHD.
ApoA-II	(17.4kda)	Primarily in HDL, enhanced hepatic lipase activity.
ApoA-IV	(46kDa)	Present in fat rich LPs.
ApoB-48	(24kDa)	Derived from ApoB-100 gene by RNA editing, found exclusively in CMs, Lack the LDLR binding domain of ApoB-100.
ApoB-100	(513kDa)	Major protein of LDL, binds to LDLR, high levels of apoB- 100 are associated with an increased risk of CAD.
ApoC-I	(7.6kDa)	Appears to be involved in activation of LCAT.
ApoC-II	(8.9kDa)	Activates LPL, deficiency of ApoC-II results in accumulation of CMs and high TG levels.
ApoC-III	(8.75kDa)	(8.75kDa) Inhibits LPL.
ApoD	(33kDa)	Found only in HDL, closely associated with LCAT.
ApoE	(34kDa	Three known apoE alleles (E2, E3, E4). Binds to LDLR, inhibits development of atherosclerosis, apoE4 is associated with late-onset Alzheimer's disease.

Apolipoprotein A1[110]

Human apoA-I circulates in plasma primarily as a component of HDL. It may be present on chylomicrons but is rarely on chylomicron remnants, VLDL or their remnants, or LDL.

Apolipoprotein A-I has two major sites of synthesis: the intestine and the liver. The intestinally derived apoA-I enters the circulation associated with chylomicrons but is rapidly transferred to HDL particles during lipase hydrolysis of chylomicrons. Hepatic apoA-I enters the circulation probably associated with nascent HDL particles having little or no core of cholesteryl ester.

The concentration of apoA-I in plasma is about 100-150 mg/dl (l), and apoA-I has a plasma half-life of about 4 days[111].

Functions of apolipoprotein A 1

Apolipoprotein A1 is the major protein component of high density lipoprotein (HDL) in plasma.

Promotes fat efflux, including cholesterol, from tissues to the liver for excretion.
It is a cofactor for lecithin cholesterol acyl transferase which is responsible for the formation of most plasma cholesteryl esters.

Apo A1 was also isolated as a prostacyclin (PGI2) stabilizing factor, and thus may have an anticlotting effect.

ApoA1 is often used as a biomarker for prediction of cardiovascular diseases and the ratio apoB- 100/apoA1 has been reported as a stronger predictor for the risk of myocardial infarction than any other lipid measurement[56].

STRUCTURE

The APOA1 gene is located on the 11th chromosome, with its specific location being 11q23-q24. The gene contains 4 exons. APOA1 encodes a 45.4 kDa protein that is composed of 396 amino acids; 21 peptides have been observed through mass spectrometry data[112].

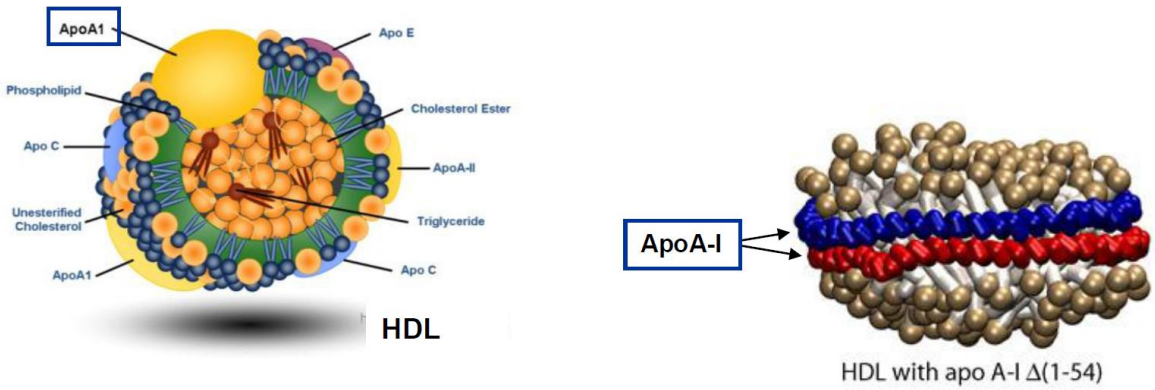


Fig 25: STRUCTURE OF APOLIPOPROTEIN A1.

Courtesy: slideshare.net

Apolipoprotein B

Apolipoprotein B (ApoB) is a protein in humans which is encoded by *APOB* gene.

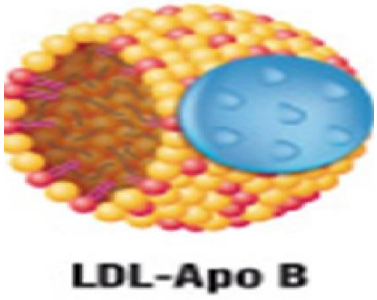


Fig 26: Structure of Apolipoprotein B.

Courtesy: Jefferydachmd.com

Apolipoprotein B is primary apolipoprotein of chylomicrons, VLDL, IDL, and LDL particles which is responsible for carrying fat molecules, including cholesterol, around the body to all cells within all tissues.

Apolipoprotein B is heterogeneous and exists penis as two forms: apoB-I00 and apoB-48. Apolipoprotein B-100 is synthesized by the liver and is an obligatory constituent of VLDL, IDL, and LDL. ApoB-48 is synthesized by the intestine and is found in chylomicrons and chylomicron remnants. Overproduction of apolipoprotein B can result in lipid-induced endoplasmic reticulum stress and insulin resistance in the liver[113].

Apolipoprotein B metabolism[114]

ApoB is made constitutively in the liver. Initiation of lipoprotein assembly requires the addition of a small amount of neutral lipid to the growing polypeptide chain. Further lipid is

added during particle assembly and secretion and the nature of the final lipoprotein released (which ranges from LDL to $VLDL_1$) depends on the extent of lipidation.

ApoB containing lipoproteins on entering the circulation are subject to lipolysis and remodeling so that the composition of the coat of the particle, especially its apoproteins, and the core change during the course of intravascular metabolism.

With elevated plasma triglycerides (HTG) produce an excess of VLDL, apoB, whereas those with raised plasma cholesterol levels overproduce $VLDL_2$ apoB[115]. IDL plus LDL apo B production is variable over the range shown and is inversely related to the plasma triglyceride concentration.

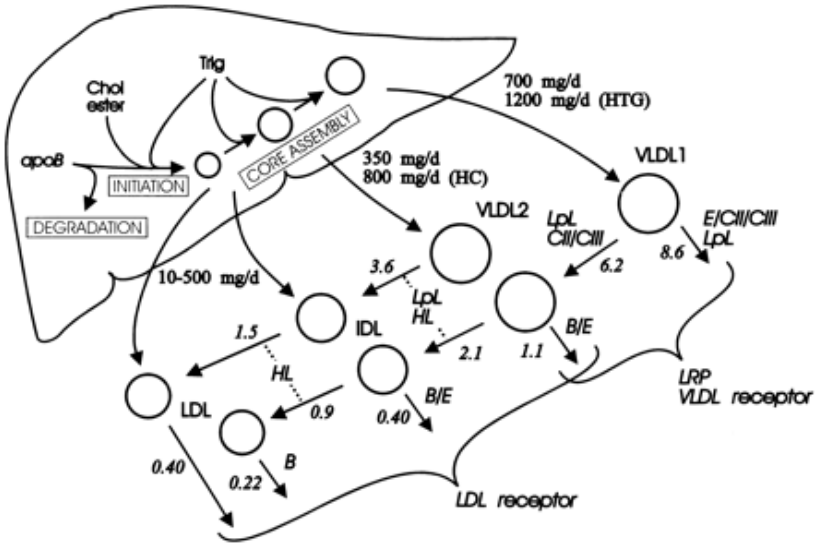


Fig 27: Apolipoprotein B metabolism.

(From the article Packard CJ, Shepherd J)

Importance of Apolipoprotien B

Apolipoprotein B is a major apolipoprotein of VLDL and LDL, the atherogenic lipoproteins. Apo B helps in solubilizing the cholesterol within the LDL complex, which in turn increases the transport capacity of LDL for subsequent deposition of cholesterol in the arterial wall. Only one molecule of Apo B exists per lipoprotein particle. The quantity of Apo B is therefore a direct measurement of VLDL and LDL particles. Due to wide variations in the amount of cholesterol in these lipoproteins, measurement of Apo B has better relevance to the concentration of atherogenic lipoprotein particles than LDL cholesterol or non-HDL cholesterol levels[116].

Lipids and Relation to atherosclerosis

In individuals with elevated plasma cholesterol levels, there is an increased incidence of atherosclerosis and its complications. It is now clear that lowering plasma cholesterol by diet and drugs slows and may even reverse the progression of atherosclerotic lesions and the complications they cause.

There is evidence that reducing plasma cholesterol may also inhibit the rupture of atherosclerotic plaques[46].

Atherogenesis

The importance of atherosclerosis as a principal cause of myocardial and cerebral infarction and thrombosis has been appreciated for many years. Nevertheless, it's cause and pathogenesis remains unsolved.

A major problem is that the disease progresses insidiously for many years before symptoms develop, making it difficult to follow the early development of the disease in individual patients, and to relate casually, the several types of lesion that have been described.

For the same reason, identification of risk factors for the disease has depended upon the relation of these factors to the clinical symptoms rather than on the extent and severity of the primary arterial lesions.

The atherosclerotic lesion in man is characterized by accumulation of lipid in and around cells of the intimal space and is associated with a cellular and fibrous proliferation, which leads to a narrowing of the lumen of the vessel.

The deposition of lipid is an early event, and the cholesterol deposited in the arterial wall is derived from plasma lipoproteins. The intima is the cell layer principally involved in atherosclerosis, although secondary changes are occasionally found in the media.

Three different types of lesions are classically recognized - the fatty streak, the fibrous plaque and the so-called complicated lesion[117]. The fatty streak, commonly found in young persons, is characterized by focal accumulation of relatively small number of intimal smooth-muscle cells, containing and surrounded by deposits of lipid.

The age at which fatty streaks appear differs in different regions of the arterial tree, but they are present in the aorta of virtually every child, regardless of race, sex or environment, by the age of 10 years. From then to the age of 25 years, the extent of aortic intimal surface covered by fatty streaks increases from about 10 % to 30 to 50 %.56

The bulk of the cholesterol found within the fatty streak probably results from imbibitions from the plasma, but it is likely also that plasma lipids are hydrolyzed and reesterified once the cells have taken them up[118].

The fibrous plaque[96, 119,120], which is the most characteristic lesion of advancing atherosclerosis, does not have the same ubiquitous distribution among the world's population that has been noted for fatty streaks. It is grossly whitish in appearance and is elevated so that it protrudes into the lumen of the artery. It consists principally of an accumulation of intimal, lipid-laden smooth-muscle cells, the lipid being primarily cholesterol and cholesteryl ester. The cells are also surrounded by lipid and by collagen, elastic fibres and proteoglycans.

Together, the cells and the extra cellular matrix components form a fibrous cap that covers a large, deeper deposit of free extra cellular lipid intermixed with cell debris[117].

The third lesion, the so-called complicated lesion, appears to be fibrous plaque that has become altered as a result of hemorrhage, calcification, cell necrosis and mural thrombosis. The distinctive characteristic of the complicated lesion is the presence of calcification. This type of lesion often becomes associated with occlusive disease.

The influence of diabetes on lipoprotein metabolism[121, 122,123,124,125,126]

Diabetes can affect all the major plasma lipoproteins as a result of insulin deficiency, insulin resistance and hyperglycemia. This may lead not only to hyperlipidaemia but also to qualitative changes in the lipoproteins, which may influence their role in atherogenesis.

VLDL in diabetes

Both increased synthesis and decreased clearance have been described and the contribution of each to hypertriglyceridemia depends on the type of diabetes and the degree of obesity, among other factors.

Lipoprotein lipase requires insulin for full activity so that in untreated insulin deficiency there is defective clearance of VLDL. This is seen in patients with IDDM or NIDDM with chronic poor control.

Insulin controls substrate flow to the liver through its effect on adipose tissue lipolysis. Lipase is inhibited by insulin so that in insulin deficiency free fatty acid concentration rise; conversely high insulin levels inhibit lipolysis.

The extent of free fatty acid mobilization determines VLDL-triglyceride production so that diabetics with raised free fatty acids tend to have increased VLDL production while those with normal free fatty acids have normal VLDL production. Diabetes thus has complex effects on the metabolisms of triglyceride, which depend on the effects of insulin on substrate flow for triglyceride metabolism. The obese NIDDM patient with mild hyperglycemia will have VLDL-triglyceride overproduction secondary to increased free fatty acid flow to the liver. In more severe NIDDM with insulin deficiency, reduced VLDL clearance is the main cause of hypertriglyceridemia.

LDL in diabetes

Insulin stimulates LDL receptor binding with subsequent internalization and degradation by the cell. Therefore insulin deficiency would lead to diminished binding of LDL and delayed clearance from the circulation.

Diabetes affect LDL metabolism by glycosylation of LDL apolipoprotein B. Glycosylation takes place at the lysine amino groups, which are important for the binding of LDL to its receptor, and so could interfere with LDL catabolism and prolong its half-life in the circulation.

HDL in diabetes[127]

During VLDL catabolism by lipoprotein lipase, surface components of the particle transfer to HDL. Therefore alterations in lipoprotein lipase activity with altered VLDL catabolism would be expected to affect plasma HDL concentrations.

HDL concentrations in diabetes correlate well with lipoprotein lipase activity. Where it is high, HDL concentrations tend to be normal or raised whereas, when lipase activity is reduced in insulin deficiency or insulin resistance, HDL levels are low and VLDL levels high.

Interpreting plasma Apo AI levels[130]

HDL is highly heterogeneous, and so apo AI in plasma resides in a variety of lipoprotein subtractions. The most abundant are larger, spherical HDL2 and HDL3. ApoA2 is present in HDL3, and to a lesser extent in HDL2. Smaller forms such as pre-b HDL can be identified on two-dimensional gel electrophoresis. They are present at low concentration but are

considered highly active. In subjects with efficient lipolysis of triglyceride-rich lipoproteins there is a low level of exchange of HDL cholesteryl ester with VLDL and chylomicrons, and so HDL remain cholesterol rich and relatively large. Individuals with effective cell efflux mechanisms will also theoretically have increased tendency to form large HDL particles. The opposite is true in hypertriglyceridaemics (with raised levels of VLDL and chylomicrons) where HDL levels are low[131].

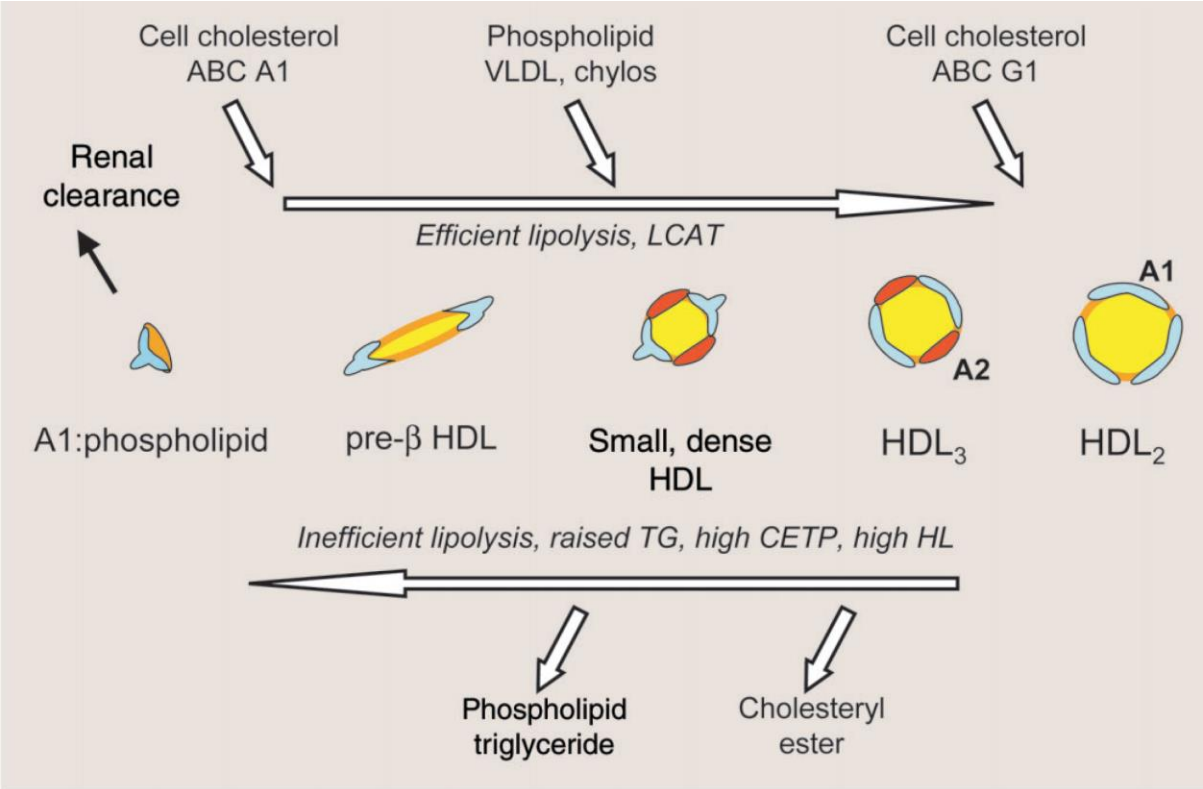


Fig 28: HDL heterogeneity.

(From the article by Marcovina S et al[130])

Interpreting plasma Apo B levels

The concentration and type of Apo B-containing lipoprotein seen in the general population and in various states of dyslipidaemia is result of the processes which regulate lipoprotein production, interconversion and clearance.

In normal subjects, although VLDL1 is the main lipoprotein secreted by the liver, its rapid progress down the dilapidation cascade means that the concentrations of VLDL1, VLDL2 and IDL in normal are kept low. LDL, is cleared relatively slowly from the circulation.

Lifestyle factors such as obesity promote VLDL synthesis which in turn leads to a rise in LDL and total Apo B[132], whilst the rise in Apo B with age is associated apparently with reduction in the activity of LDL receptors[133]. VLDL1 production is also influenced by insulin levels even in apparently healthy subjects. In people with insulin resistance there is a failure to regulate properly VLDL production, and Apo B levels rise in the VLDL, IDL and LDL density classes[132].

In Individuals with significant hypercholesterolemia (LDL C >4.5 mmol L) are likely to have raised levels of all Apo B-containing lipoproteins as a result of reduced clearance of particles by receptors. The LDL receptor is a regulated cell membrane protein responsible for the internalization of lipoproteins by cells that require cholesterol.

In individuals with hypertriglyceridemia there is a combination of overproduction of VLDL (especially VLDL1) and inefficient lipolysis. Thus, Apo B levels are elevated in both VLDL1 and VLDL2. Furthermore, a prolonged residence time in the circulation is seen for all Apo B-

containing lipoproteins which increases the opportunities for these particles to be modified by the processes of inter-particle exchange of lipid and protein components, and by lipolysis.

Elevation of apo B in VLDL is therefore associated not only with a higher number of particles but also with the fact that these lipoproteins have altered comparison. Remnant particles are particularly atherogenic as they can cause directly cholesterol deposition in macrophages.

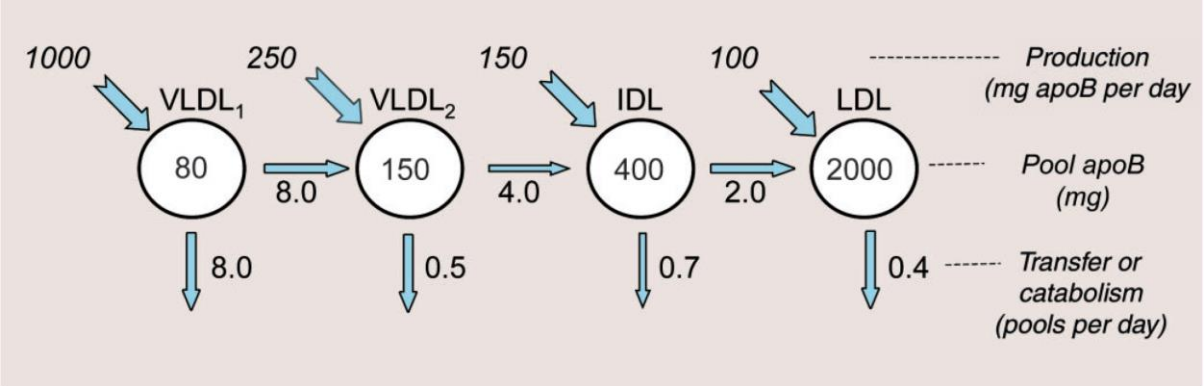


Fig 29: Metabolic regulation of plasma and lipoprotein apo B levels.

(From the article by Marcovina S et al[130])

(Depicted within each circle is the circulating pool (in mg) of apo B in VLDL1, VLDL2, IDL and LDL in normolipidaemic subjects. Rates of production by the liver for the four major classes are given in mg apo B per day. Fractional rates of conversion, e.g. from VLDL1 to VLDL2 (8.0 pools per day) or of catabolism (e.g. for LDL 0.4) pools per day are also provided.)

Management of Dyslipidemia in Diabetes mellitus[123, 128,129]

Individuals with Diabetes mellitus may have several forms of Dyslipidemia. Because of the additive cardiovascular risk of hyperglycemia and hyperlipidaemia, lipid abnormalities should be aggressively detected and treated.

The Heart protection study suggests that in people with diabetes over the age of 40years with total cholesterol > 135 mg/dl, an LDL reduction of ∼30% regardless of baseline LDL levels is appropriate. These recommendations are based not only on the high incidence of coronary heart disease in patients with diabetes, but also on the higher case fatality rate of these patients once they have cardiovascular disease.

Based on the guidelines provided by American Diabetes Association and the American Heart Association, the order of priorities in the treatment of hyperlipidaemia is

I. **LDL Cholesterol lowering**

• Life style interventions

• Preferred

-HMG CoA reductase inhibitors

• Others

-Bile acid binding resins

-Fenofibrate or Niacin

II. **HDL cholesterol raising**

• Life style interventions

• Nicotinic acid or Fibrates

III. **Triglyceride lowering**

• Life style interventions

• Glycemic control

• Fabric acid derivatives

• Niacin

• High dose statins (in those who have high LDL cholesterol)

IV. Combined hyperlipidaemia

• First choice

Improve glycemic control + high dose statin

- Second choice

  Improve glycemic control + statin + fibric acid derivative

- Third choice

  Improve glycemic control + statin + nicotinic acid

Screening for Dyslipidemia

In all adults aged 20 years or older, a fasting lipoprotein profile should be obtained once every 5 years.

In diabetics, test for lipid disorders at least annually and more often if needed to achieve goals.

In diabetics with low-risk lipid values (LDL < 100 mg/dl, HDL > 50mg/dl and Triglycerides < 150mg/dl), repeat lipid assessments every two years.

MATERIALS AND METHODS

SOURCE OF DATA

One hundred and thirty patients who were diagnosed to be having Type 2 Diabetes mellitus, both out patients and in patients admitted in R L Jalappa Hospital satisfying the aforementioned criteria were included in the study.

Sample size was estimated by difference in mean.

Using the formula:

$$\text{Sample size} = 2SD2\ (Z\alpha/2+Z\beta)\ 2/d2$$

SD-Standard deviation = from previous studies or pilot study

$Z\alpha/2 = Z0.05/2 = Z0.025 = 1.96$ (from Z table) at type 1 error of 5%

$Z\beta = Z0.20 = 0.84$ (from Z table) at 80% power.

d = effect size = difference between mean values.

Alpha at 20%

Power of 80%

n=59+59 in each group

With 10% non-response rate

n=118+11.8=130 cases

n=65+65 in each group

Sample size- 65 in study group (Diabetics with microvascular complications)

65 in control group (Diabetics without microvascular complications)

METHOD OF COLLECTION OF DATA

Study sample (n) = 130

Study Group 1 (n) = 65(diabetes mellitus + microvascular complications)

1a - diabetic neuropathy (n) = 20±5

1b - diabetic retinopathy (n) = 20±5

1c - diabetic nephropathy (n) = 20±5

Control Group (n) = 65 (diabetes mellitus without microvascular complications)

A detailed history was taken and recorded according to the proforma.

The patients was subjected to complete clinical examination including clinical tests for assessment of peripheral neuropathy, funduscopic examination by ophthalmologist for assessment of retinopathy and laboratory investigations for nephropathy.

Patient was explained about the entire procedure and informed consent was taken in his/her own understandable language.

Under aseptic precautions about 5 ml of blood was drawn from the medial cubital vein after minimum of 8 hours of fasting for fasting blood sugars and lipid profile.

Post prandial blood sugars was analyzed after 2 hours of their regular unchanged breakfast/diet and medication if any. HbA1C, complete haemogram was analyzed irrespective of fasting, post prandial or random sample.

For blood glucose estimation plasma, Ethylene Diamine Tetra Acetic Acid (EDTA), fluoride tube was used to avoid / inhibit enolase enzyme and glycolysis. Serum sample was used lipid profile, blood urea, and serum creatinine.

Fig 30: Vacutainers used for collecting blood samples of patients.

(Sodium fluoride with EDTA, plain FOR, plain EDTA)

For complete haemogram and HbA1C whole blood was used. For whole blood and serum, plain vacutainer was used.

Blood glucose estimation is done by glucose oxidase and peroxidase method using dry chemistry analyzer. Lipid profile will be analyzed using lipid chemistry auto analyzer method and standardized procedure. Lipoprotein (a), apolipoprotein A and apolipoprotein B was estimated by immunoturbidometric method.

HbA1C is estimated by the gold slandered High Performance Liquid Chromatography (HPLC) method. Urine microalbumin was estimated using random spot sample.

Biothesiometry was performed on all patient who had clinical suspicion on clinical examination of neuropathy and neuropathy severity was categorized according to biothesiometer findings.

Fig 31: Biothesiometer

Random blood sugar was done first to see whether patient has diabetes or not then later FBS, PPBS was done to confirm the diagnosis.

Laboratory Investigation methodology

Serum Lipid Profile

Is analyzed using vitros 250 anlyzer which works on the principle of refractive photometry.

Total Cholesterol (TC) [135]

Methodology – Modified Roeschalau's Method

Principle:-Cholesterol is oxidized to cholesterol-4-en-3-one and hydrogen peroxide (H_2O_2). This H_2O_2 reacts with the chromogenic substrate 4 Aminoantipyrine (4AAP) to form a colored complex quinoneimine by peroxidase. The peroxidase intensity of the colour formed is directly proportional to concentration of cholesterol which is read at 505 nm.

The estimation of Cholesterol involves the following enzyme catalyzed reaction.

1. Cholesterol ester $\xrightarrow{CE}$ Cholesterol + Fatty Acid.
2. Cholesterol + O_2 $\xrightarrow{CHOD}$ Cholest-4-en-3-one + H_2O_2.
3. H_2O_2 + 4AAP + Phenol $\xrightarrow{PEROXIDASE}$ $2H_2O$ + Quinoneimine.

CE – Cholesterol Esterase

4AAP – 4 Aminoantipyrine

CHOD – Cholesterol Oxidase

Triglycerides[136]

Methodology:-GPO-Trinder method, end point Wako and modified Me Gowan et al and Fossati et al.

Principle:-Triglycerides are hydrolysed by lipase to release glycerol which is phosphorylated to Glycerol-3-Phospate. Glycerol-3-phosphate is oxidided to Dihydroxy acetone phosphate (DAP) and H_2O_2. This H_2O_2 oxidizes a chromogenic substance 4 aminoantipyrine (4AAP) to give quinoeimine by peroxidase. The intensity of chromogen formed is directly proportional to triglyceride concentration and is measured at 505nm.

Triglycerides + H_2O $\xrightarrow{\text{LIPASE}}$ Glycerol + Free Fatty Acid.

Glycerol + ATP $\xrightarrow{\text{GLYCEROL KINASE}}$ Glycerol-3-Phospate + ADP.

Glycerol-3-Phospate + O_2 $\xrightarrow{\text{GLYCEROL PHOSPHATE OXIDASE}}$ DAP + H_2O_2.

H_2O_2+ 4AAP + 3, 5-DHBS $\xrightarrow{\text{PEROXIDASE}}$ Quinoeimine dye + 2 H_2O.

High Density Lipoprotein (HDL) [137]

Principle: The reaction between cholesterol other than HDL and enzyme for cholesterol assay is suppressed by the electrostatic interaction between polyanions and cationic substances. Hydrogen peroxide is formed by the free cholesterol in HDL by cholesterol oxidase. Oxidative condensation of EMSE and 4–AA is caused by hydrogen peroxide in the

presence of peroxidase and the absorbants of the resulting red-purple quinine is measured to obtain the cholesterol value in HDL.

Other lipoproteins than HDL $\xrightarrow{\text{POLYANIONS}}$ Suppresses reaction with enzyme cationic substance.

HDL (cholesterol esters) + H_20 $\xrightarrow{\text{CHOLESTEROL ESTERASE}}$ HDL (free cholesterol) + Free fatty acids.

HDL (free cholesterol) + O_2 + H^+ $\xrightarrow{\text{CHOLESTEROL OXIDASE}}$ Cholestenone + H_2O_2.

$2H_20_2$ + 4-AA + EMSE + H_3 + O $\xrightarrow{\text{PEROXIDASE}}$ Red – purple quinine + 5 H2O.

EMSE: N - Ethyl - N – (3-methylphenyl) – N'succinylethyenediame.

4 – AA: 4 – Aminoantipyrine.

Very Low Density Lipoprotein (VLDL) Cholesterol[138]

It is calculated by the following formula.

VLDL in mg/dl = Triglycerides /5

Low Density Lipoprotein (LDL) Cholesterol[138]

It is calculated by the Freidewald formula.

LDL in mg/dl= Total cholesterol - HDL – TG/5

Apoprotein A-I (Quantia Apo A-I) [139]

Apo A-I is a turbidimetric immunoassay for the determination of apolipoprotein AI and is based on the principle of agglutination reaction. The test specimen is mixed with quantia Apo AI activation buffer (R1) and antibody reagent (R2) and allowed to react. Presence of Apo AI in the test specimen results in formation of an insoluble complex

resulting in an increase in turbidity, which is measured at wavelength 340 nm. The increase in turbidity corresponds to the concentration of Apo AI in the test specimen.

Apoprotein B (Quantia Apo B) [140]

Apo B is a turbidimetric immunoassay for the determination of apolipoprotein B and is based on the principle of agglutination reaction. The test specimen is mixed with quantia Apo B activation buffer (R1) and quantia Apo B antibody reagent (R2) and allowed to react. Presence of Apo B in the test specimen results in formation of an insoluble complex resulting in an increase in turbidity, which is measured at wavelength 340 nm. The increase in turbidity corresponds to the concentration of Apo B in the test specimen.

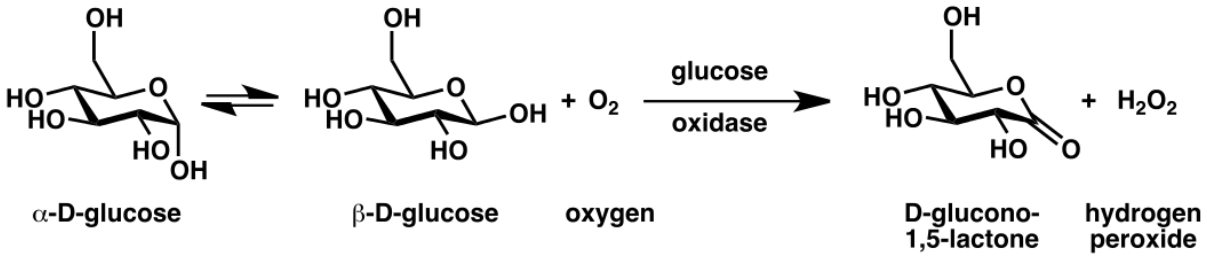
Fig 32: Lp(a), Apo A1 and Apo B was done with vitros 5.1 (fusion) analyzer.

RBS, FBS AND PPBS

Analyzed using dry chemistry automated analyzer (vitros 250 analyzer) using glucose oxidase enzymatic method.

Principle of procedure

The glucose oxidase enzyme (GOx) also known as notatin is an oxido reductase that catalyzes the oxidation of glucose to hydrogen peroxide and D-glucono-δ-lactone.



Mechanism

At pH 7, glucose exists in solution in cyclic hemiacetal form as 63.6% β-D-glucopyranose and 36.4% α-D-glucopyranose, the proportion of linear and furanose form being negligible. The glucose oxidase binds specifically to β-D-glucopyranose and does not act on α-D-glucose. It is able to oxidise all of the glucose in solution because the equilibrium between the α and β anomers is driven towards the β side as it is consumed in the reaction.[3]

Glucose oxidase catalyzes the oxidation of β-D-glucose into D-glucono-1,5-lactone, which then hydrolyzes to gluconic acid.

In order to work as a catalyst, GOx requires a cofactor, flavin adenine dinucleotide (FAD). FAD is a common component in biological oxidation-reduction (redox reactions). Redox reactions involve a gain or loss of electrons from a molecule. In the GOx-catalyzed redox reaction, FAD works as the initial electron acceptor and is reduced to FADH2. Then FADH2 is oxidized by the final electron acceptor, molecular oxygen (O2), which can do so because it has a higher reduction potential. O2 is then reduced to hydrogen peroxide (H2O2).

Glucose oxidase coupled with peroxidase reaction that visualizes colorimetrically formed H2O2, determine free glucose in sera or blood plasma using spectrometric assays manually or with automated procedures, and even point of use rapid assays.[3][7]

Fig 33: RBS, FBS, PPBS and Lipid profile done using Vitros 250 analyzer.

HbA1C

Was analyzed by using Bio Rad D10 analyzer using principle of high performance liquid chromatography.

Fig 34: Bio Rad D-10 analzer used for Hba1C.

INCLUSION CRITERIA

1. Patients more than 30 years of age

2. Patients fulfilling ADA criteria for diagnosis of diabetes mellitus.

3. Patients having microvascular complications such as diabetic retinopathy, diabetic neuropathy, and diabetic nephropathy.

ADA Criteria for diagnosis of diabetes mellitus:

- FBS ≥126mg/dl, no caloric intake for 8hours or
- HBA1C ≥6.5%; or
- Random plasma glucose ≥200 mg/dl with symptoms of diabetes mellitus.

EXCLUSION CRITERIA

1. Patients with chronic liver disease;

2. Patients who are known Type I DM

3. Patients who were already on lipid lowering drugs.

4. Females taking Oral contraceptive pills, HRT, Niacin, Corticosteroids.

STUDY DESIGN

It is a Hospital based Comparative cross sectional study

STATISTICAL METHODS

Descriptive and inferential statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean ± SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5 % level of significance. The following assumptions on data is made,

Assumptions: 1.

Dependent variables should be normally distributed, Two Samples drawn from the population should be random, Cases of the samples should be independent Student t test (two tailed, independent) has been used to find the significance of study parameters on continuous scale between two groups (Inter group analysis) on metric parameters.

Chi-square/ Fisher Exact test has been used to find the significance of study parameters on categorical scale between two or more groups, Non-parametric setting for Qualitative data analysis.

Significant figures

+ Suggestive significance (P value: $0.05<P<0.10$)

* Moderately significant (P value: $0.01<P \leq 0.05$)

** Strongly significant (P value: $P \leq 0.01$)

Statistical software: The Statistical software namely SAS 9.2, SPSS 15.0, Stata 10.1, MedCalc 9.0.1 ,Systat 12.0 and R environment ver.2.11.1 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc**.**

OBSERVATION AND RESULTS

Table 4: Age distribution of patients studied.

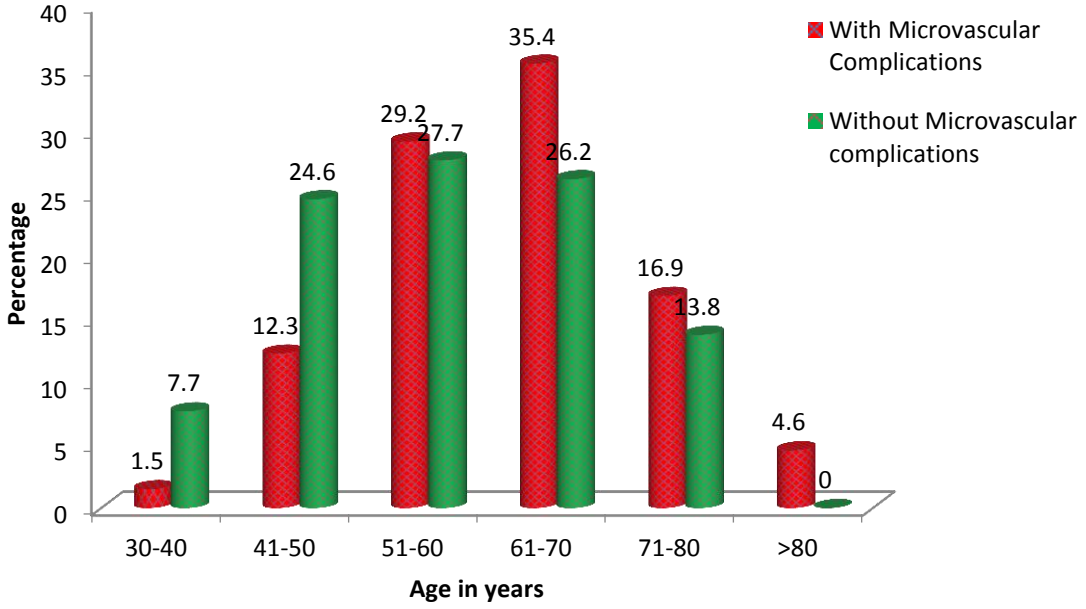
Age in years	With Microvascular Complications	Without Microvascular complications	Total
30-40	1(1.5%)	5(7.7%)	6(4.6%)
41-50	8(12.3%)	16(24.6%)	24(18.5%)
51-60	19(29.2%)	18(27.7%)	37(28.5%)
61-70	23(35.4%)	17(26.2%)	40(30.8%)
71-80	11(16.9%)	9(13.8%)	20(15.4%)
80-90	3(4.6%)	0(0%)	3(2.3%)
Total	65(100%)	65(100%)	130(100%)
Mean ± SD	63.51±10.51	57.60±11.82	60.55±11.53

P=0.003**, significant, Student t test

In this study total no of patients were 65, in which 65 patients were diabetic with microvascular complication (study group) and 65 patients were diabetics without microvascular complication (control group).

Our study patients age was beween 30 - 90 yrs and average age of patients in patients with microvascular complicatin was 63.51±10.51yrs and patients without microvascular complication was 57.60±11.82 yrs which is statistically significant.

In our study group maximum no of patients were in the age group of 61-70yrs i.e 23 (35.4%) patients and in control group were in the age group of 51-60yrs i.e 18 (27.7%).



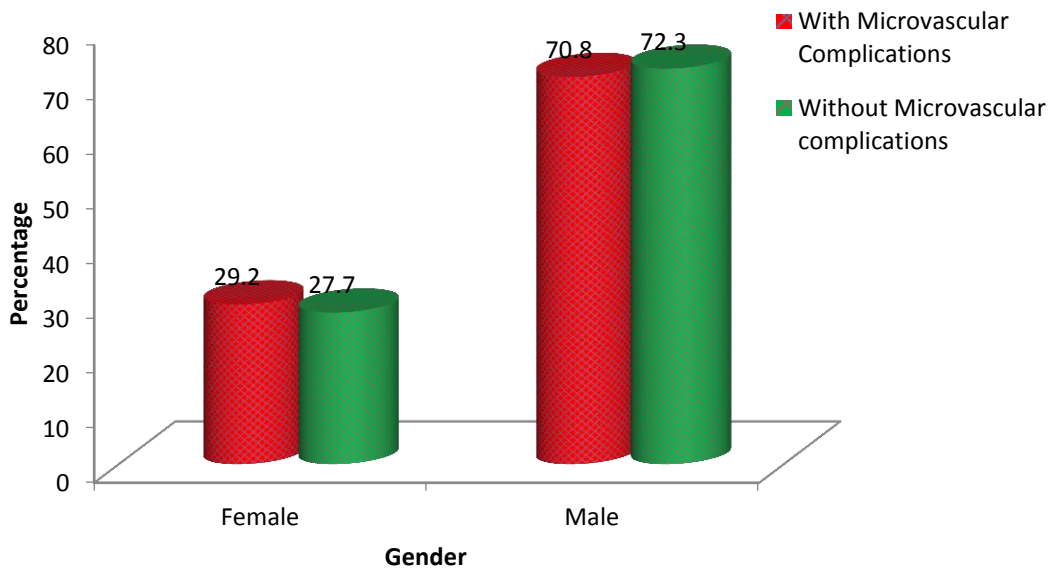
Graph 1: Bar diagram showing age distribution (in percentage).

Table 5: Gender distribution of patients studied.

Gender	With Microvascular Complications	Without Microvascular complications	Total
Female	19(29.2%)	18(27.7%)	37(28.5%)
Male	46(70.8%)	47(72.3%)	93(71.5%)
Total	65(100%)	65(100%)	130(100%)

P=0.846, Not significant, Chi-Square test

Gender distribution in study is 46(70.8%) were males and 19(29.9%) were females in patients with microvascular complication and 47(72.3%) were females and 18(27.7%) were males. P value =0.846.



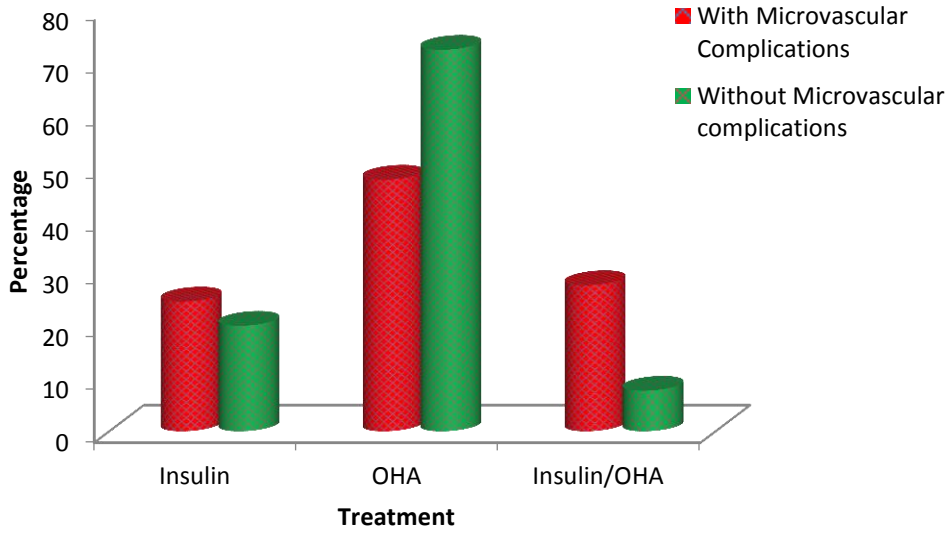
Graph 2: Bar diagram showing gender distribution (in percentage).

Table 6: Treatment distribution in two groups of patients studied.

Treatment	With Microvascular Complications	Without Microvascular complications	Total
Insulin	16(24.6%)	13(20%)	29(22.3%)
OAD	31(47.7%)	47(72.3%)	78(60%)
Insulin/OAD	18(27.7%)	5(7.7%)	23(17.7%)
Total	65(100%)	65(100%)	130(100%)

P=0.004**, Significant, Chi-Square test

Maximum no of patients 31(47.7%) and 47(72.3%) were taking OAD's with microvascular complication and without microvascular complication respectively. 18(27.7%) and 5(7.7%) were on both insulin/OAD. 16(24.6%) and 13(20%) were only on insulin with and without microvascular complication respectively. P value was 0.004 and was significant



Graph 3: Bar diagram showing treatment distribution among the patients.

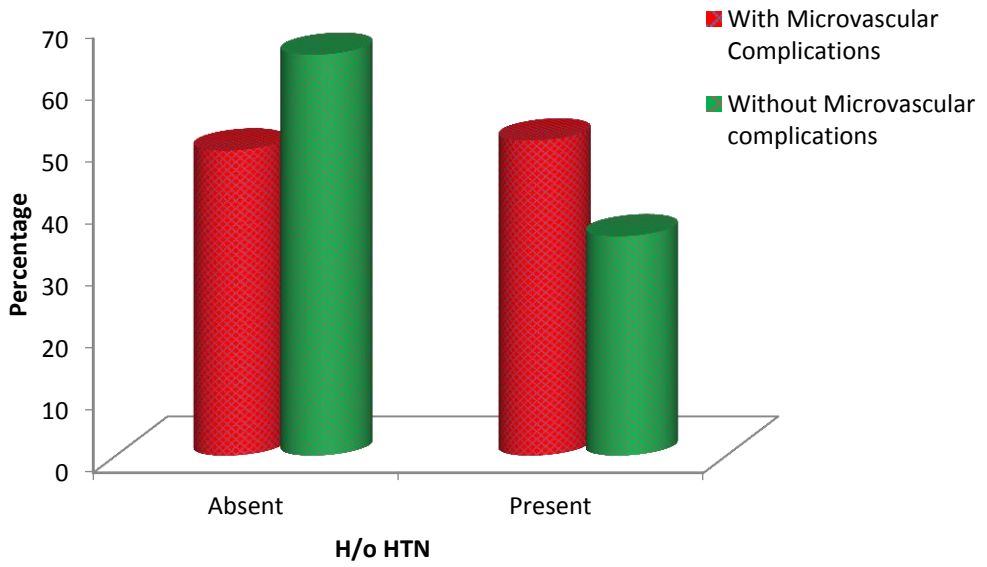
Table 7: H/o HTN incidence in two groups of patients studied

H/o HTN	With Microvascular Complications	Without Microvascular complications	Total
Absent	32(49.2%)	42(64.6%)	74(56.9%)
Present	33(50.8%)	23(35.4%)	56(43.1%)
Total	65(100%)	65(100%)	130(100%)

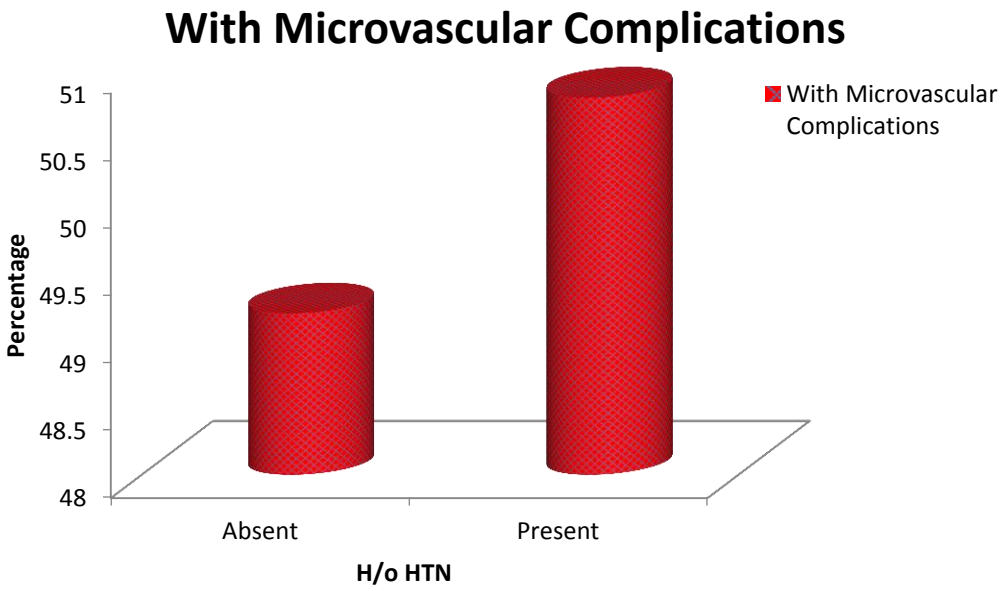
P=0.077+, Significant, Chi-Square test

Incidence of hypertension was 33(50.8%) and 23(35.4%) with and without microvascular complication and is statistically significant P value is 0.007.

Implies that patients with microvascular complication higher incidence of hypertension.



Graph 4: Bar diagram showing HTN incidence in two groups of patients studied.



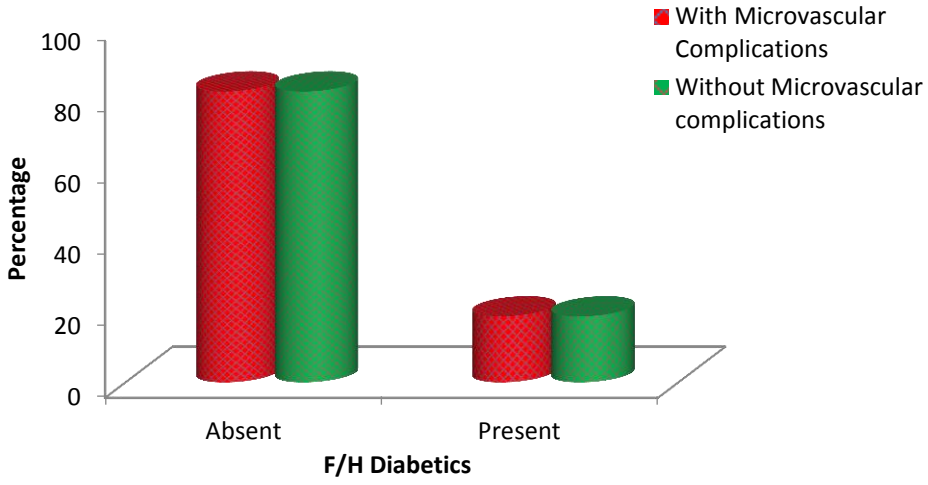
Graph 5: Bar Diagram showing incidence of HTN in patients with microvascular complications.

Table 8: F/H Diabetics Incidence in two groups of patients studied.

F/H Diabetics	With Microvascular Complications	Without Microvascular complications	Total
Absent	53(81.5%)	53(81.5%)	106(81.5%)
Present	12(18.5%)	12(18.5%)	34(26.2%)
Total	65(100%)	65(100%)	130(100%)

P=1.000, Not Significant, Chi-Square test

Incidence of family history of diabetes was 12(18.5%) both same in patients with and without microvascular complication. This implies that family history of diabetes is similar in both the patients.



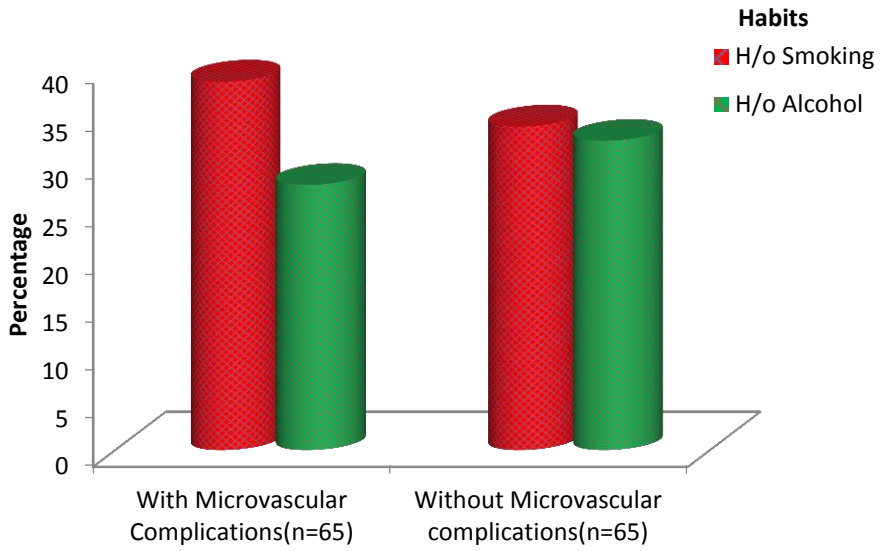
Graph 6: Bar diagram showing Incidence of family history of diabetes in two groups of patients studied.

Table 9: Habits distribution in two groups of patients studied.

Habits	With Microvascular Complications (n=65)	Without Microvascular complications (n=65)	Total (n=130)	P value
H/o Smoking	25(38.5%)	22(33.8%)	47(36.2%)	0.584
H/o Alcohol	18(27.7%)	21(32.3%)	39(30%)	0.566

Chi-Square test/fisher exact test

25(38.5%) out of 65 patients had history of smoking with microvascular complication and 22(33.8%) out of 65 patients without microvascular complication. P value was 0.584 18(27.7%) out of 65 patients hat history of alcohol intake with microvascular complication and 21(32.3%) patients had history of alcohol intake without microvascular complication. P value was 0.566



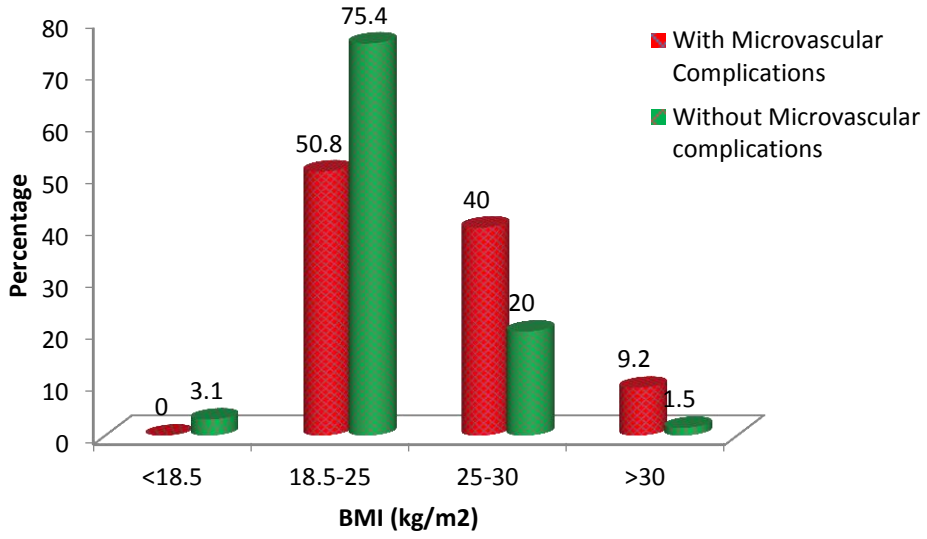
Graph 7: Bar diagram showing Habits distribution in two groups of patients studied.

Table 10: BMI (kg/m^2) distribution in two groups of patients studied.

BMI (kg/m^2)	With Microvascular Complications	Without Microvascular complications	Total
<18.5	0(0%)	2(3.1%)	2(1.5%)
18.5-25	33(50.8%)	49(75.4%)	82(63.1%)
25-30	26(40%)	13(20%)	39(30%)
>30	6(9.2%)	1(1.5%)	7(5.4%)
Total	65(100%)	65(100%)	130(100%)
Mean ± SD	25.74±3.25	23.51±2.80	24.62±3.22

P<0.001**, Significant, student t test

Mean BMI in our patient with microvascular complication was 25.74±3.25 kg/m2 and mean BMI without microvascular complication was 23.51±2.80 kg/m2. Which is statistically significant P<0.001 Most of the patients with and without microvascular complication, BMI was between 18.5-25 kg/m^2. Implies that most of the patients with diabetes are obese.



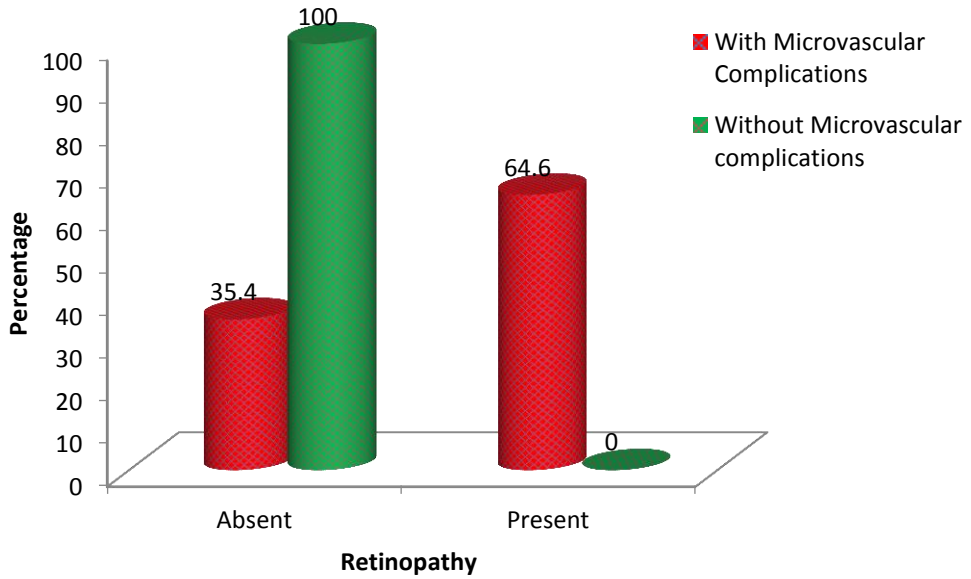
Graph 8: Bar diagram showing percentage distribution of BMI in kg/m^2.

Table 11: Retinopathy incidence in two groups of patients studied.

Retinopathy	With Microvascular Complications	Without Microvascular complications	Total
Absent	23(35.4%)	65(100%)	88(67.7%)
Present	42(64.6%)	0(0%)	42(32.3%)
Total	65(100%)	65(100%)	130(100%)

P<0.001**, Significant, Chi-Square test

42(64.6%) out of 65 patients had diabetic retinopathy in patients with microvascular complications and statistically significant.P<0.001



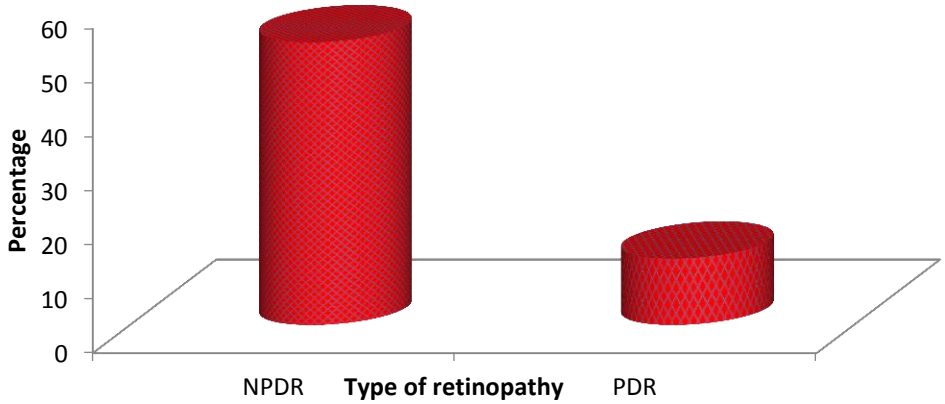
Graph 9: Bar diagram showing Retinopathy incidence in two groups of patients studied.

Table 12: Type of retinopathy in patients with Microvascular complications.

Type of retinopathy	No of patients	%
NPDR	34	80.9
PDR	8	19.0
Total	42	100.0

Out of 42 patients who had diabetic retinopathy 34(80.9%) patients had non proliferative diabetic retinopathy and 8 (19%) patients had proliferative diabetic retinopathy.

This implies that patients with diabetes most common type of retinopathy seen is non proliferative diabetic retinopathy.



Graph 10: Bar diagram showing Type of retinopathy in patients with Microvascular complications.

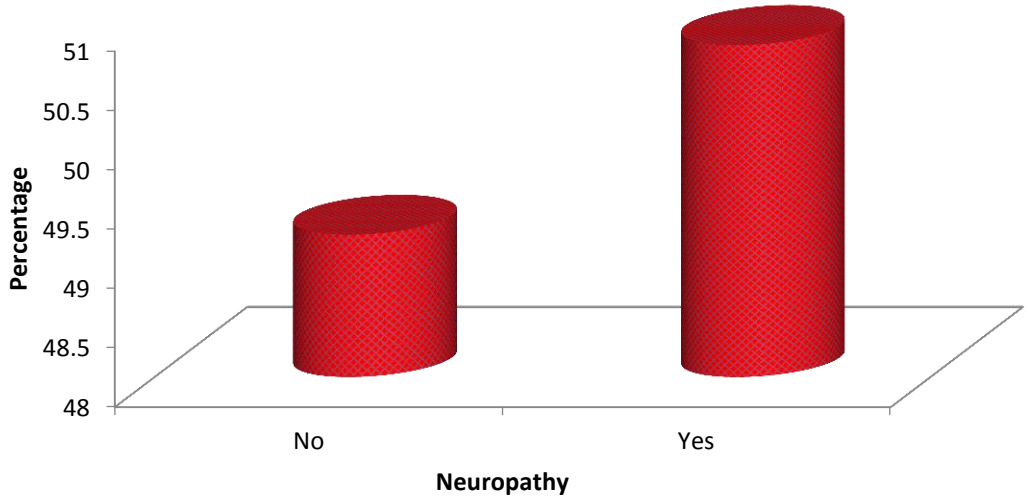
Table 13: Incidence of Nephropathy and Neuropathy in patients studied.

	No. of patients (n=65)	**%**
Nephropathy		
• No	15	23.1
• Yes	50	76.9
Neuropathy		
• No	32	49.2
• Yes	33	50.8

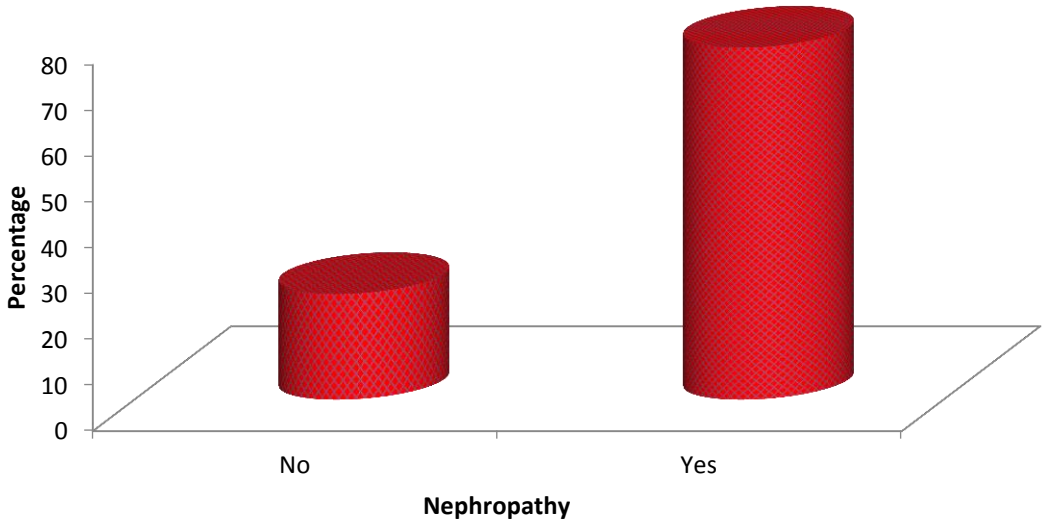
In this study 50 (76.9%) had diabetic nephropathy and 33 (50.8%) had diabetic neuropathy out of 65 patients.

This implies that incidence of diabetic nephropathy was more than diabetic retinopathy than diabetic neuropathy.

In this study most of the patients had diabetic nephropathy which is the commonest complication after that diabetic retinopathy.



Graph 11: Bar diagram showing incidence of diabetic neuropathy.

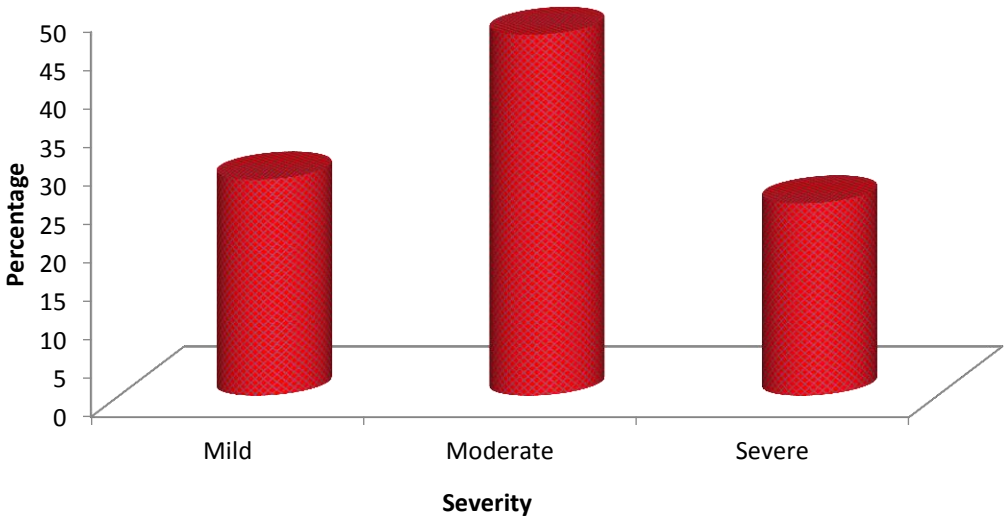


Graph 12: Bar diagram showing incidence of diabetic nephropathy.

Table 14: Severity of neuropathy distribution.

Severity	No. of patients	%
Mild	9	28.1
Moderate	15	46.9
Severe	8	25.0
Total	32	100.0

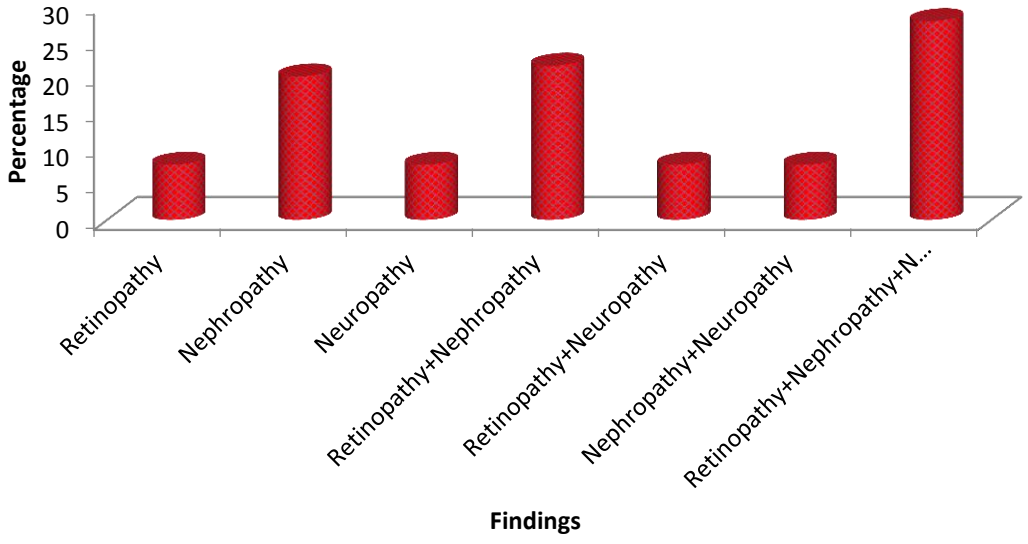
15(46.9%) had moderate diabetic neuropathy out of 32 patients.



Graph 13: Bar diagram showing Severity of neuropathy distribution.

Table 15: Distribution of incidence of retinopathy, neuropathy and nephropathy.

Findings	No of patients (n=65)	%
Retinopathy	5	7.7
Nephropathy	13	20.0
Neuropathy	5	7.7
Retinopathy+Nephropathy	14	21.5
Retinopathy+Neuropathy	5	7.7
Nephropathy+Neuropathy	5	7.7
Retinopathy+Nephropathy+Neuropathy	18	27.7



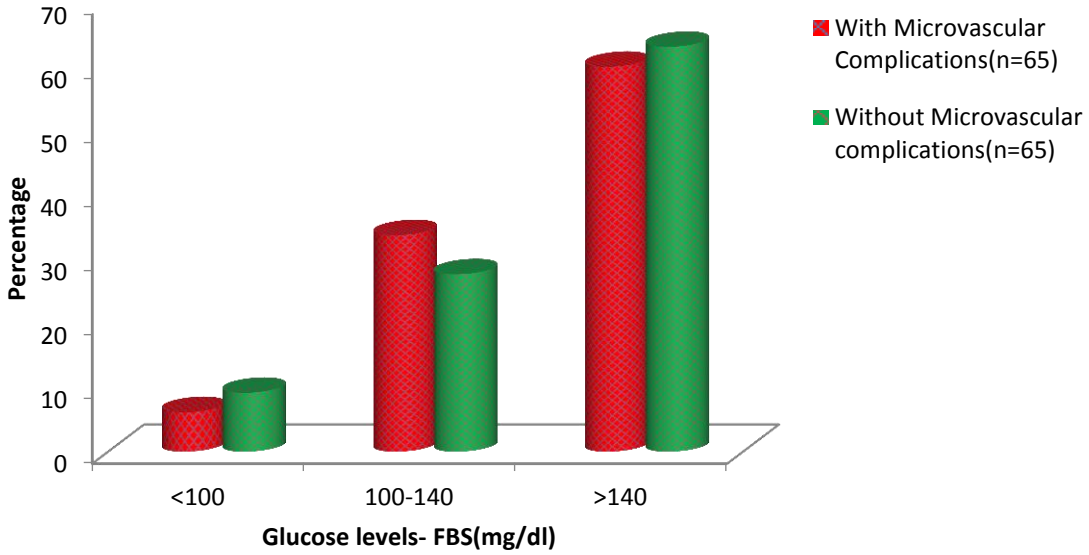
Graph 14: Bar diagram showing Distribution of incidence of retinopathy, neuropathy and nephropathy.

Table 16: Glucose parameters in two groups of patients studied.

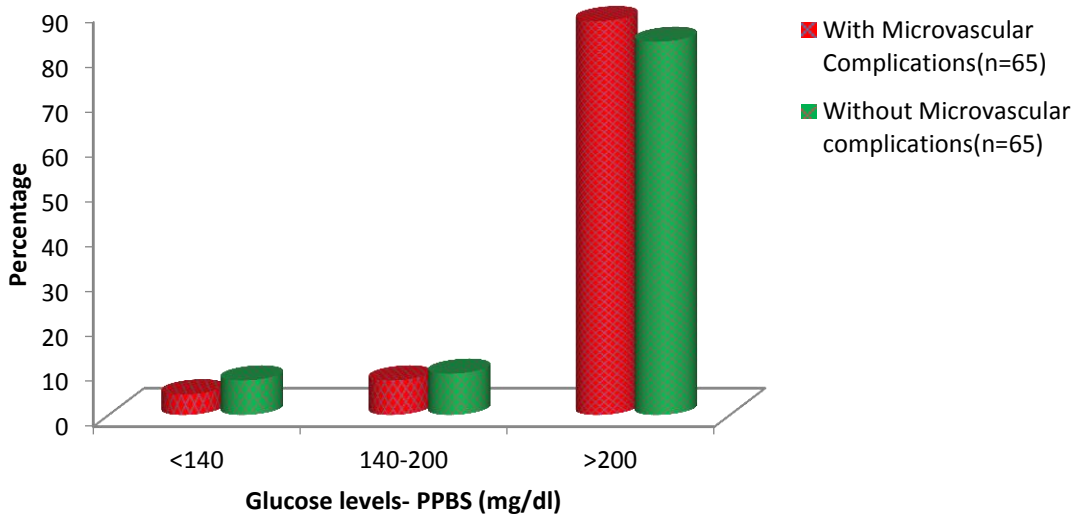
Glucose levels	With Microvascular Complications (n=65)	Without Microvascular complications (n=65)	Total (n=130)	P value
FBS (mg/dl)				
• <100	4(6.2%)	6(9.2%)	10(7.7%)	0.654
• 100-140	22(33.8%)	18(27.7%)	40(30.8%)	
• >140	39(60%)	41(63.1%)	80(61.5%)	
PPBS (mg/dl)				
• <140	3(4.6%)	5(7.7%)	8(6.2%)	0.761
• 140-200	5(7.7%)	6(9.2%)	11(8.5%)	
• >200	57(87.7%)	54(83.1%)	111(85.4%)	
HbA1c%				
• <6	0(0%)	7(10.8%)	7(5.4%)	<0.001**
• 6-9	38(58.5%)	45(69.2%)	83(63.8%)	
• >9	27(41.5%)	13(20%)	40(30.8%)	

Chi-Square test/Fisher Exact test

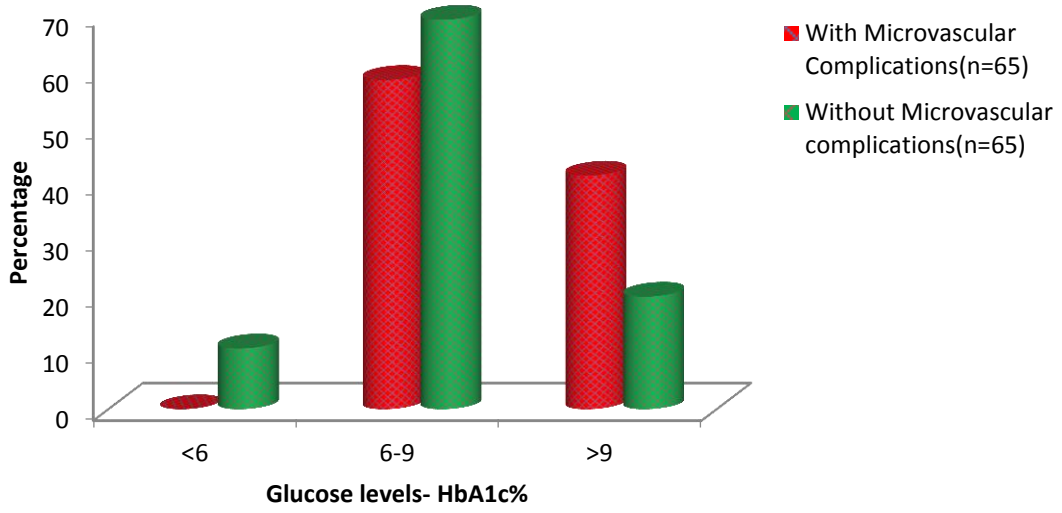
In this study patients with microvascular complication had poor glycemic control than the patients without microvascular complications. Most of the Patients with microvascular complication had poor glycemic control.



Graph 15: Bar diagram showing FBS levels in patients studied.



Graph 16: Bar diagram showing PPBS levels in patients studied.



Graph 17: Bar diagram showing HbA1C levels in patients studied.

Table 17: Comparison of Glucose variables in two groups of patients studied.

variables	With Microvascular Complications	Without Microvascular complications	Total	P value
RBS	175.97±52.36	125.38±58.18	150.68±60.70	<0.001**
FBS (mg/dl)	152.65±40.11	149.15±41.59	150.90±40.73	0.627
PPBS (mg/dl)	270.62±69.38	251.57±70.24	261.09±70.19	0.122
HbA1c%	8.76±1.71	7.88±1.97	8.32±1.89	0.007**

Student t test

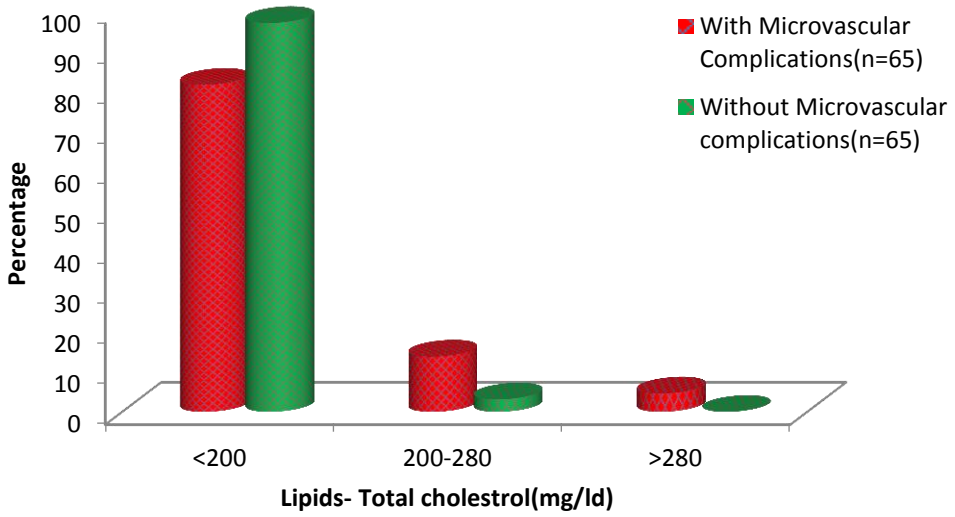
Mean RBS of patients was 175.97±52.36 mg/dl and 125.38±58.18 mg/dl with and without microvascular complication P<0.001. Mean FBS of patients was 152.65±40.11mg/dl and 149.15±41.59 mg/dl with and without microvascular complication P value 0.627. Mean PPBS was 270.62±69.38 mg/dl and 251.57±70.24 mg/dl with and without microvascular complication p value 0.122. Mean HbA1c was 8.76±1.71% and 7.88±1.97% with and without microvascular complication P value 0.007.

In this study the RBS, FBS, PPBS and HbA1c were abnormally elevated and patients with microvascular complication has poor glycemic control than patients without microvascular complications.

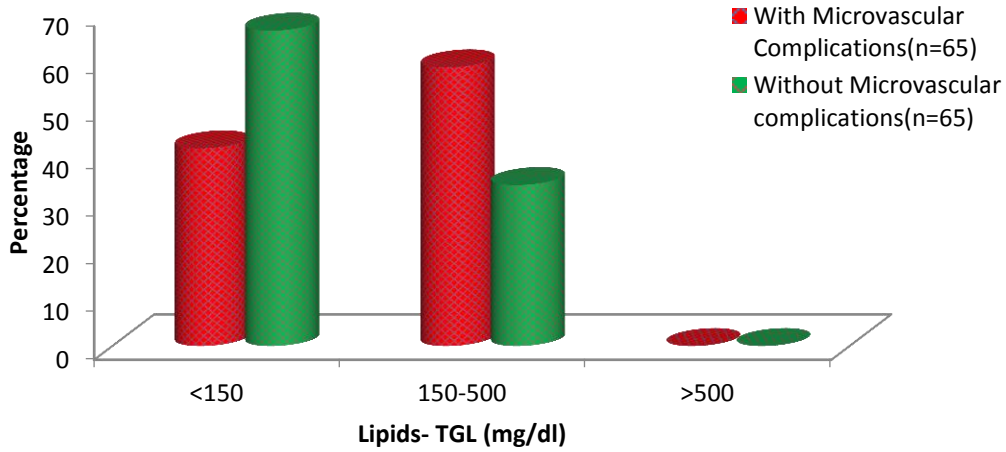
Table 18: Lipids distribution in two groups of patients studied.

Lipids	With Microvascular Complications (n=65)	Without Microvascular complications (n=65)	Total (n=130)	P value
Total Cholesterol (mg/dl)				
• <200	53(81.5%)	63(96.9%)	116(89.2%)	0.013*
• 200-280	9(13.8%)	2(3.1%)	11(8.5%)	
• >280	3(4.6%)	0(0%)	3(2.3%)	
TGL (mg/dl)				
• <150	27(41.5%)	43(66.2%)	70(53.8%)	0.008**
• 150-500	38(58.5%)	22(33.8%)	60(46.2%)	
• >500	0(0%)	0(0%)	0(0%)	
HDL (mg/dl)				
• <35	31(47.7%)	24(36.9%)	55(42.3%)	0.301
• 35-60	30(46.2%)	39(60%)	69(53.1%)	
• >60	4(6.2%)	2(3.1%)	6(4.6%)	
LDL (mg/dl)				
• <70	9(13.8%)	11(16.9%)	20(15.4%)	0.270
• 70-190	51(78.5%)	53(81.5%)	104(80%)	
• >190	5(7.7%)	1(1.5%)	6(4.6%)	
VLDL (mg/dl)				
• <35	35(53.8%)	57(87.7%)	92(70.8%)	<0.001**
• 35-60	25(38.5%)	8(12.3%)	33(25.4%)	
• >60	5(7.7%)	0(0%)	5(3.8%)	

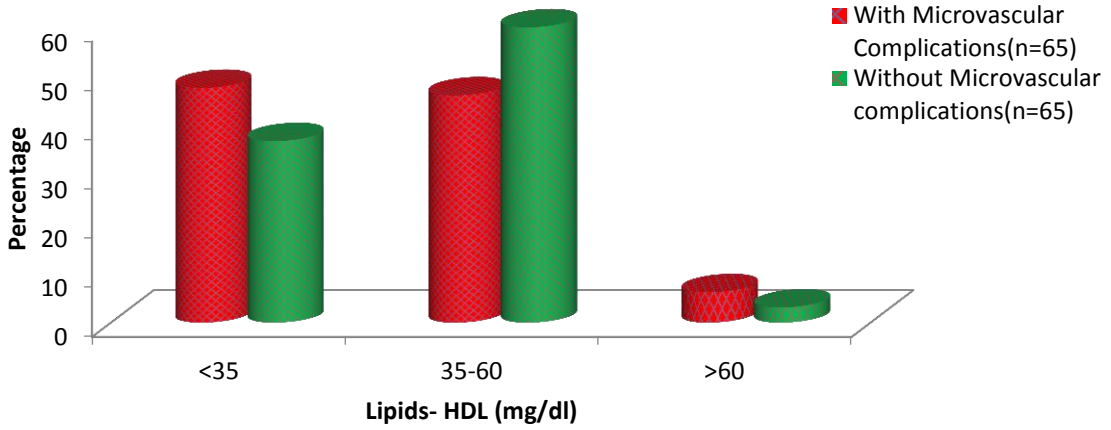
Chi-Square test/Fisher Exact test



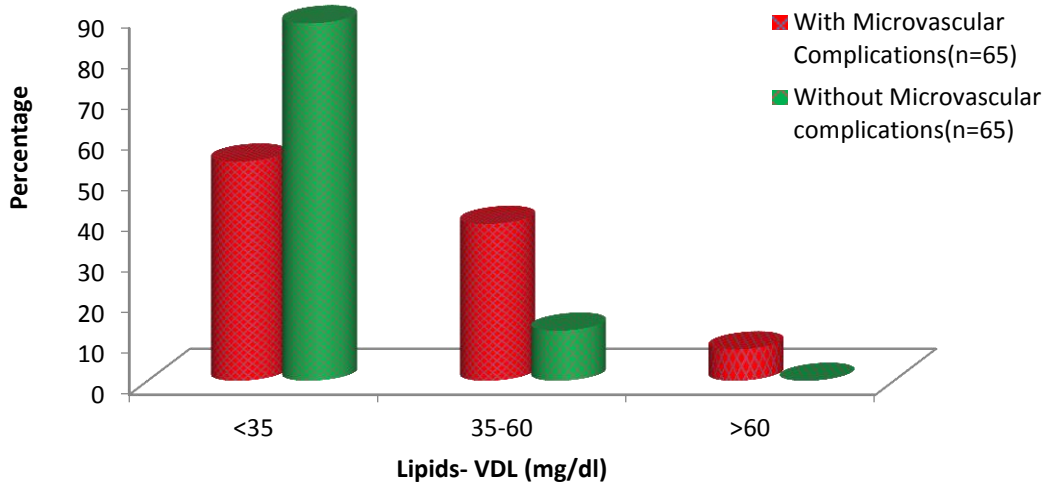
Graph 18: Bar diagram showing total cholesterol distribution in two groups of patients studied.



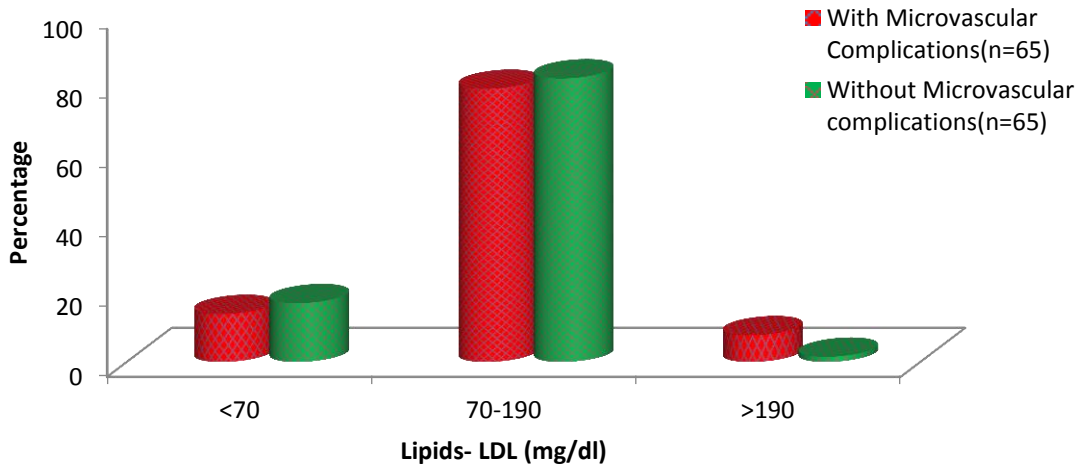
Graph 19: Bar diagram showing triglyceride distribution in two groups of patients studied.



Graph 20: Bar diagram showing HDL distribution in two groups of patients studied.



Graph 21: Bar diagram showing VLDL distribution in two groups of patients studied.



Graph 22: Bar diagram showing LDL distribution in two groups of patients studied.

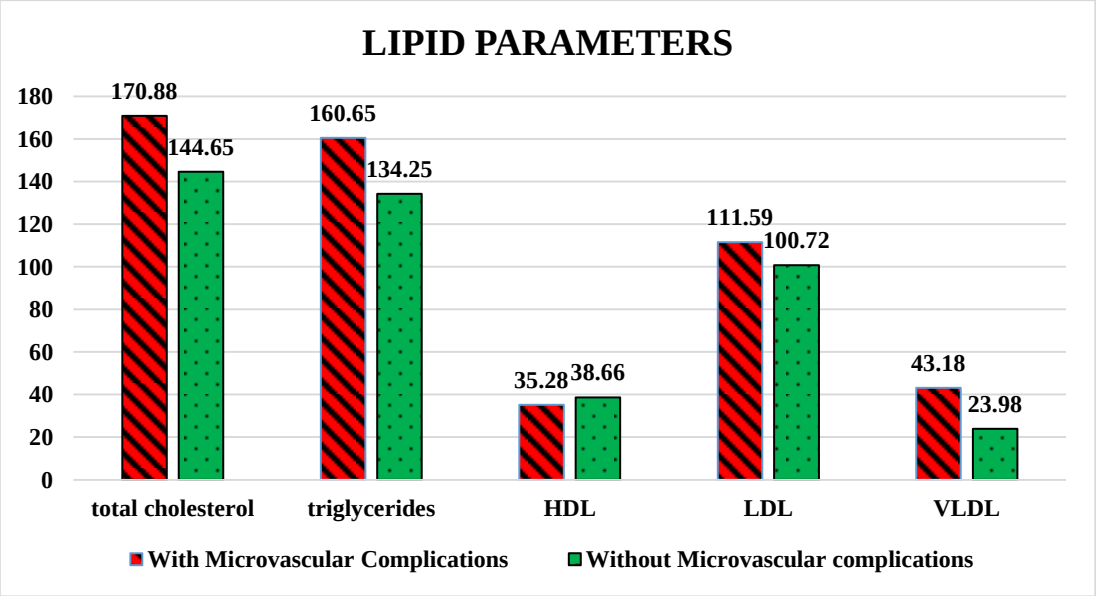
Table 19: Comparison of Lipid parameters in two groups of patients studied.

Lipids	With Microvascular Complications	Without Microvascular complications	Total	P value
Total Cholesterol (mg/dl)	170.88±53.03	144.65±35.38	157.76±46.79	<0.001**
TGL (mg/dl)	160.65±55.12	134.25±44.61	147.45±51.67	0.003**
HDL (mg/dl)	35.28±12.18	38.66±10.76	36.97±11.57	0.096+
LDL (mg/dl)	111.59±44.16	100.72±36.19	106.16±40.59	0.127
VLDL (mg/dl)	43.18±54.36	23.95±10.38	33.56±40.16	0.006**

Student t test

In present study total cholesterol, triglycerides, LDL, VLDL is higher in patients with microvascular complication without microvacular complication.

HDL was lower in patients with microvascular complication



Graph 23: Bar diagram showing mean traditional lipid parameters in mg/dl.

Y axis showing levels of lipid parameters in mg/dl and x axis showing different lipid parameters

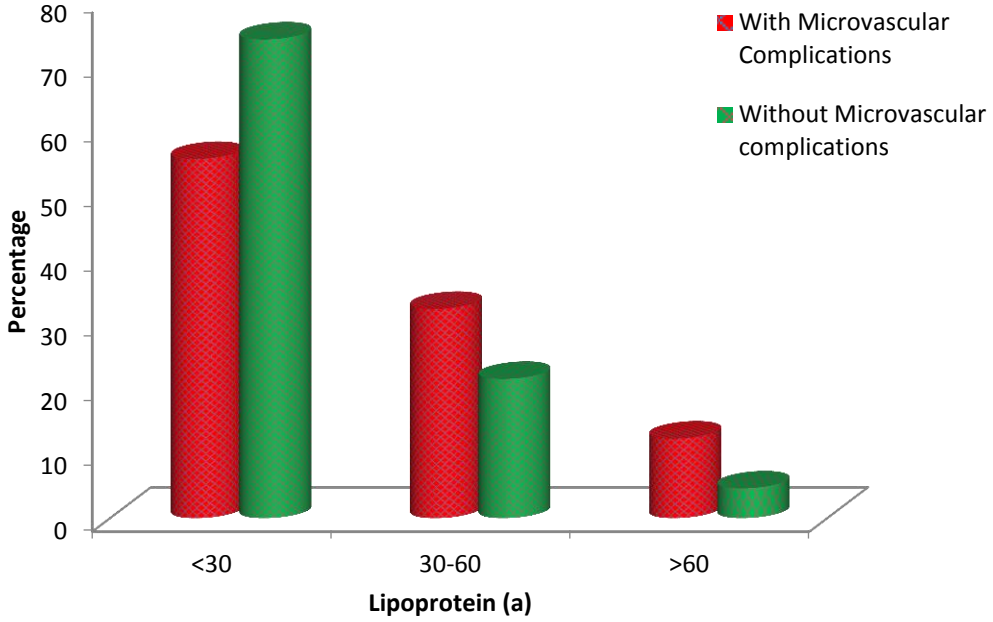
Table 20: Lipoprotein (a) distribution in two groups of patients studied.

Lipoprotein (a)	With Microvascular Complications	Without Microvascular complications	Total
<30	36(55.4%)	48(73.8%)	84(64.6%)
30-60	21(32.3%)	14(21.5%)	35(26.9%)
>60	8(12.3%)	3(4.6%)	11(8.5%)
Total	65(100%)	65(100%)	130(100%)
Mean ± SD	32.30±22.30	24.62±16.61	28.46±19.96

P=0.028*, Significant, student t test

In my patients mean lipoprotein (a) level are 32.30±22.30 mg/dl and 24.62±16.61mg/dl with and without microvascular complication P value 0.028. and is statistically significant.

In my study lipoprotein (a) levels was higher with microvascular complication than without microvascular complication and significantly higher.

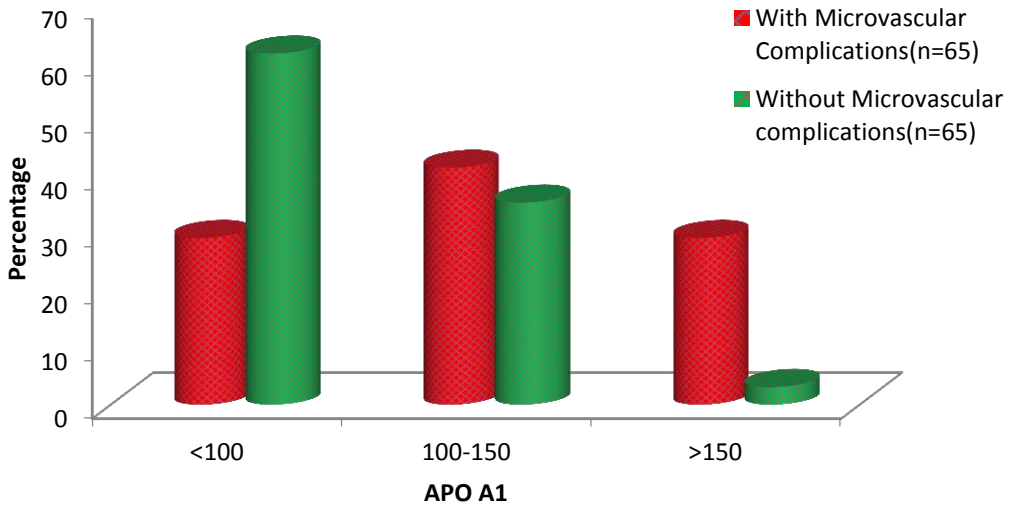


Graph 24: Bar diagram showing mean lipoprotein levels in mg/dl.

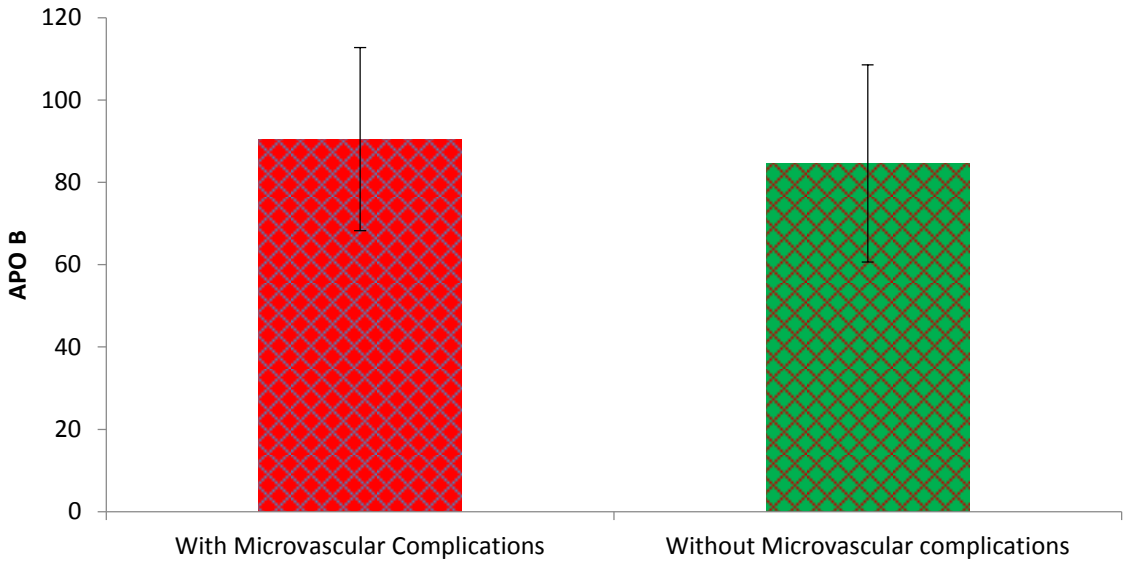
Table 21: APO-A1 and APO-B distribution in two groups of patients studied.

APO	With Microvascular Complications (n=65)	Without Microvascular complications (n=65)	Total (n=130)	P value
APO A1				
• <100	19(29.2%)	40(61.5%)	59(45.4%)	<0.001**
• 100-150	27(41.5%)	23(35.4%)	50(38.5%)	
• >150	19(29.2%)	2(3.1%)	21(16.2%)	
APO B				
• <80	23(35.4%)	33(50.8%)	56(43.1%)	0.164
• 80-140	38(58.5%)	28(43.1%)	66(50.8%)	
• >140	4(6.2%)	4(6.2%)	8(6.2%)	

Chi-square test/Fisher Exact test



Graph 25: Bar diagram showing Apo A1 distribution in patients with and without microvascular complication.



Graph 26: Bar graph showing Apo B in patients with and without microvascular complication.

Table 22: Comparison of Lipoprotein, APO-A1 and APO-B in two groups of patients studied.

	With Microvascular Complications	Without Microvascular complications	Total	P value
Lipoprotein (a) mg/dl	32.30±22.30	24.62±16.61	28.46±19.96	0.028*
APO A1 mg/dl	123.42±41.96	90.85±28.13	107.13±39.16	<0.001**
APO B mg/dl	90.49±22.26	84.60±23.94	87.55±23.21	0.149

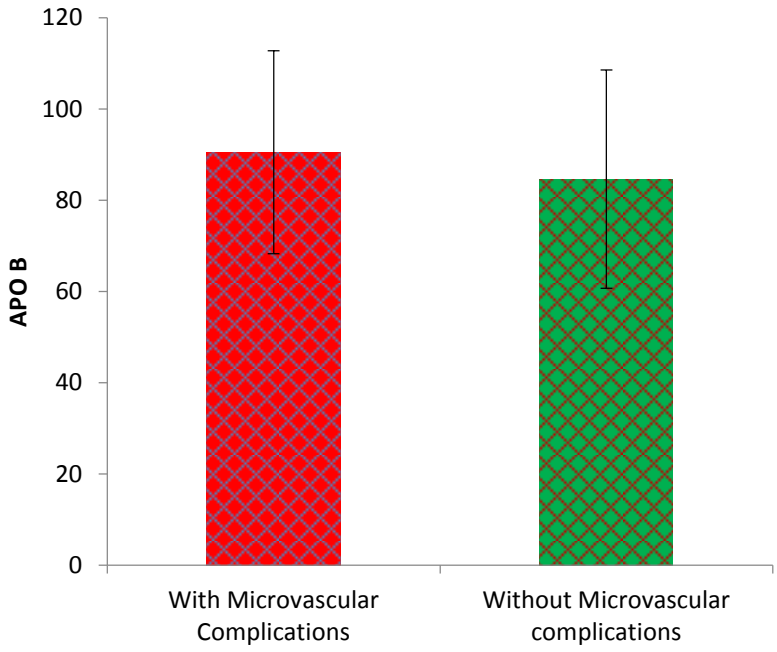
Student t test

In this study mean lipoprotein (a) levels were 32.30±22.30 and 24.62±16.61 mg/dl with and without microvascular complications respectively and statistically significant.

Mean Apo A1 levels were 123.42±41.96 and 90.85±28.13 mg/dl with and without microvascular complication respectively and statistically significant.

Mean Apo B levels were 90.49±22.26 and 84.60±23.94 mg/dl with and without microvascular complication respectively and not statistically significant.

This implies that patients with microvascular complications had higher levels of Lp (a), Apo A1 and Apo B than without microvascular complication.



Graph 27: Bar diagram showing mean Apo B levels in mg/dl.

Table 23: Comparison of Lipid parameters in relation to incidence of retinopathy.

Lipids	Retinopathy		Total	P value
	Absent	Present		
Total Cholesterol (mg/dl)	169.83±53.93	171.45±53.18	170.88±53.03	0.907
TGL (mg/dl)	161.30±47.03	160.29±59.62	160.65±55.12	0.944
HDL (mg/dl)	36.83±12.22	34.43±12.22	35.28±12.18	0.452
LDL (mg/dl)	113.47±38.73	110.57±47.29	111.59±44.16	0.802
VLDL (mg/dl)	36.87±14.10	46.63±66.87	43.18±54.36	0.493

Student t test

In this study patients with retinopathy had high VLDL levels compared to patients without retinopathy.

Other lipids levels were little higher with retinopathy than without retinopathy but not statistically significant.

Table 24: Comparison of Lipid parameters in relation to incidence of Nephropathy.

Lipids	Nephropathy		Total	P value
	Absent	Present		
Total Cholesterol (mg/dl)	168.6±49.03	178.47±54.44	170.88±53.03	0.532
TGL (mg/dl)	157.78±48.39	176.87±56.52	160.65±55.12	0.196
HDL (mg/dl)	33.47±10.33	35.82±12.72	35.28±12.18	0.516
LDL (mg/dl)	92.73±31.03	117.25±46.16	111.59±44.16	0.059+
VLDL (mg/dl)	28.15±13.37	47.69±60.98	43.18±54.36	0.225

Student t test

Patients with nephropathy had higher levels of total cholesterol, triglycerides, LDL, VLDL compered to patients without microvascular complications.

HDL levels were higher in patients with nephropathy than without nephropathy.

Table 25: Comparison of Lipid parameters in relation to incidence of Neuropathy.

Lipids	Neuropathy		Total	P value
	Absent	Present		
Total Cholesterol (mg/dl)	175.72±63.22	166.18±41.31	170.88±53.03	0.473
TGL (mg/dl)	156.63±52.67	164.55±57.93	160.65±55.12	0.567
HDL (mg/dl)	37.13±14.56	33.48±9.20	35.28±12.18	0.231
LDL (mg/dl)	107.70±45.00	115.36±43.69	111.59±44.16	0.489
VLDL (mg/dl)	36.04±15.47	50.10±74.69	43.18±54.36	0.301

Student t test

In this study mean levels of total cholesterol, Triglycerides, LDL, VLDL levels were higher in patients with neuropathy than patients without neuropathy.

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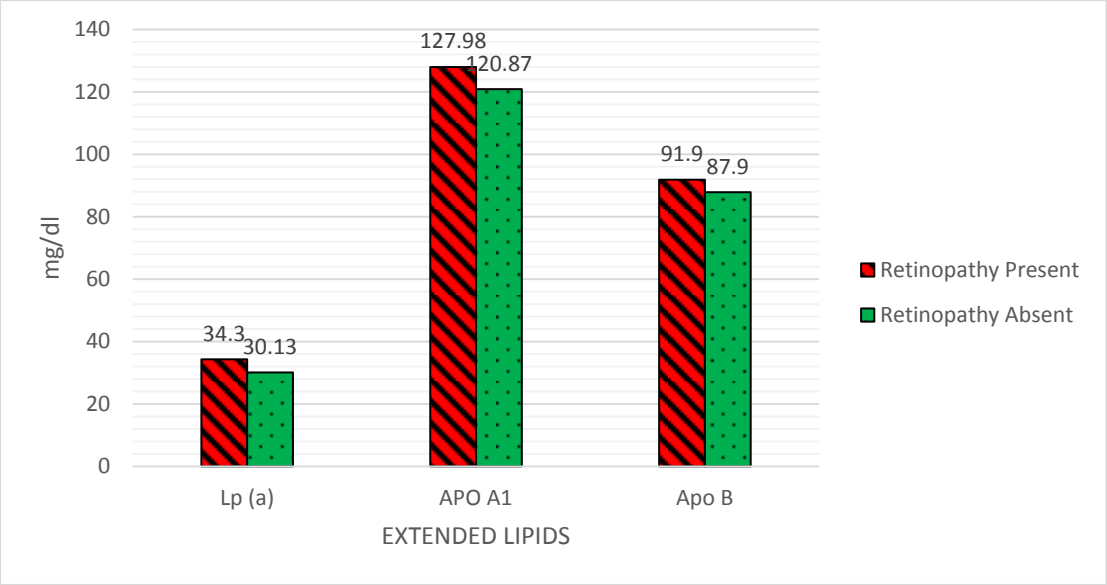
Table 26: Comparison of Lipoprotein (a), APO-A1 and APO-B in relation to incidence of retinopathy.

Variables	Retinopathy		Total	P value
	Absent	Present		
Lipoprotein (a) mg/dl	30.13±20.84	34.30±23.24	32.30±22.30	0.629
APO A1 mg/dl	120.87±34.81	127.98±45.62	123.42±41.96	0.531
APO B mg/dl	87.91±21.64	91.90±22.72	90.49±22.26	0.494

Student t test

In this study mean Lp (a) levels were 34.30±23.24 and 30.13±20.84 mg/dl, mean Apo A1 levels were 127.98±45.62 and 120.87±34.81 mg/dl and mean Apo B levels were 91.90±22.72 and 87.91±21.64 mg/dl with and without retinopathy respectively.

Inference: patients with retinopathy have elevated levels of all extended lipids which are comparably higher in patients with retinopathy than without retinopathy but not abnormally elevated and not statistically significant.



Graph 28: Bar diagram showing mean extended lipid levels in diabetic retinopathy patients.

Table 27: Comparison of Lipo protein, APO-A1 and APO-B in relation to incidence of Nephropathy.

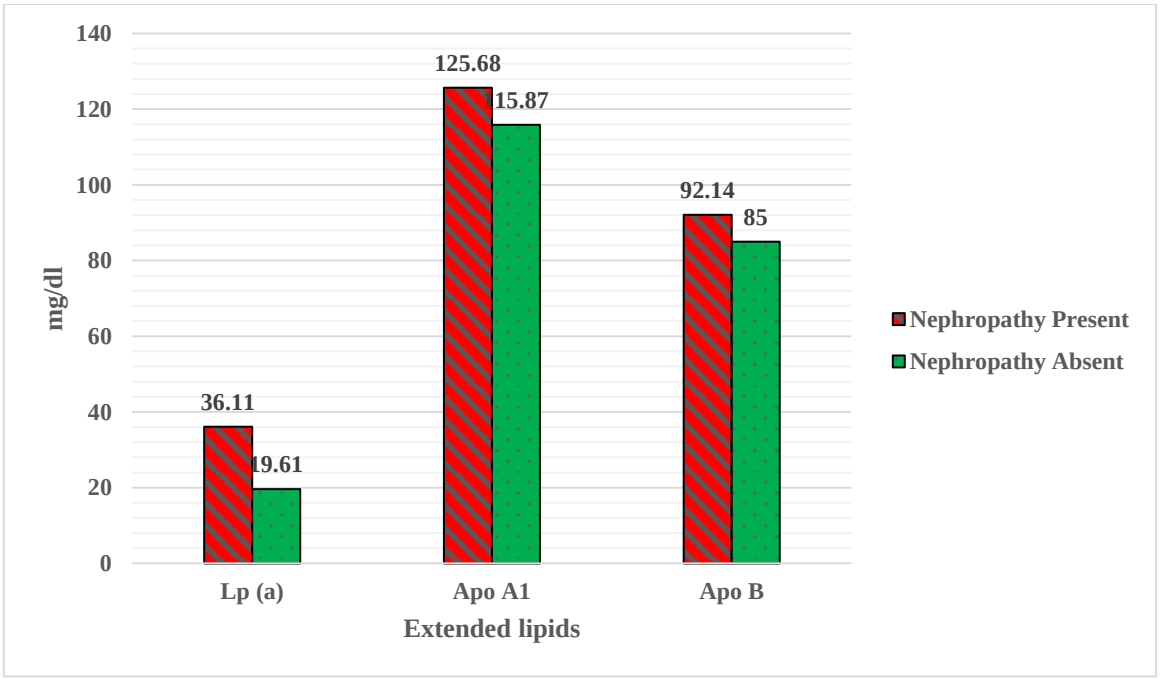
Variables	Nephropathy		Total	P value
	Absent	Present		
Lipoprotein (a) mg/dl	19.61±11.90	36.11±23.34	32.30±22.30	0.011*
APO A1 mg/dl	115.87±42.52	125.68±41.96	123.42±41.96	0.431
APO B mg/dl	85.00±20.00	92.14±22.82	90.49±22.26	0.279

Student t test

In this study mean Lp(a) levels were 36.11±23.34 and 19.61±11.90 mg/dl, mean Apo A1 levels were 125.68±41.96 and 115.87±42.52 mg/dl and mean Apo B levels were 92.14±28.82 and 85.00±20.00 mg/dl with and without nephropathy respectively .

Inference: patients with nephropthy have elevated levels of all extended lipids which are comparably higher in patients with nephropathy than without nephropathy.

Lp(a) levels was statistically significant.



Graph 29: Bar diagram showing mean extended lipid levels in diabetic nephropathy patients.

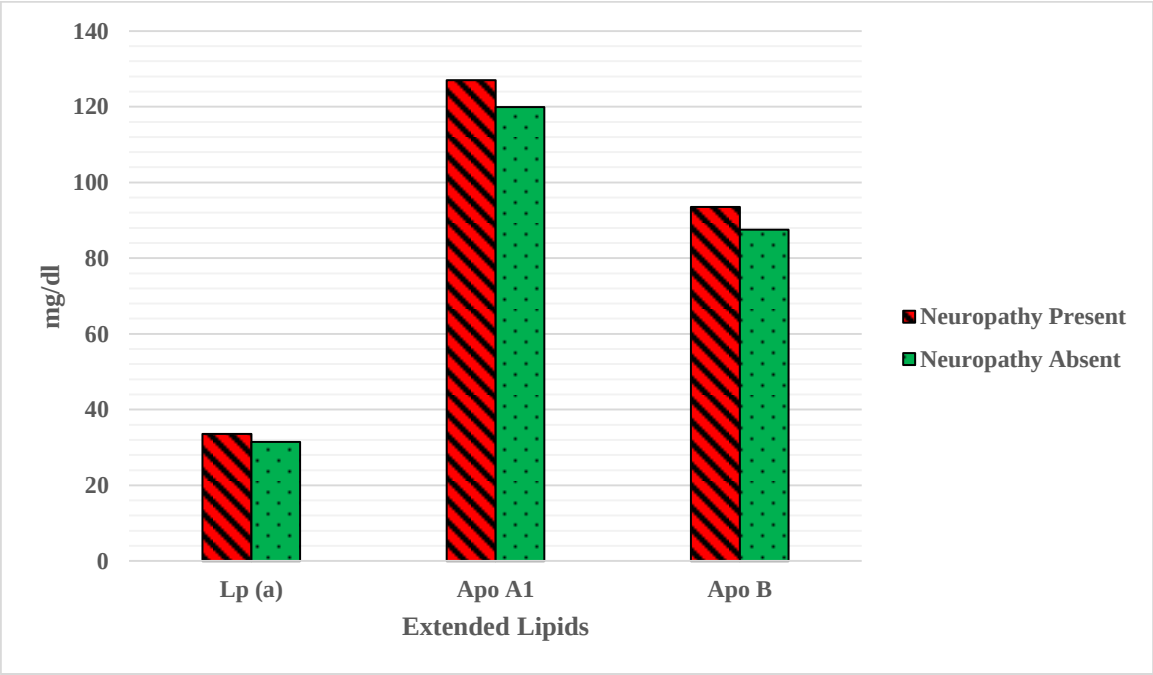
Table 28: Comparison of Lipoprotein, APO-A1 and APO-B in relation to incidence of Neuropathy.

variables	Neuropathy		Total	P value
	Absent	Present		
Lipoprotein (a) mg/dl	31.46±23.62	33.16±21.19	32.30±22.30	0.761
APO A1 mg/dl	119.94±44.32	127.00±39.76	123.42±41.96	0.502
APO B mg/dl	87.55±18.11	93.53±25.79	90.49±22.26	0.282

Student t test

In this study mean Lp (a) levels were 33.16±21.19and 31.46±23.62 mg/dl, mean Apo A1 levels were 127.00±39.76 and 119.94±44.32 mg/dl and mean Apo B levels were 93.53±25.79 and 87.55±18.11 mg/dl with and without neuropathy respectively.

Inference: patients with neuropathy have elevated levels of all extended lipids which are comparably higher in patients with neuropathy than without neuropathy but not abnormally elevated and not statistically significant.



Graph 30: Bar diagram showing mean extended lipid levels in diabetic neuropathy patients.

DISCUSSION

Type 2 Diabetes Mellitus is one of the common medical illness and serious metabolic disorder which is rapidly raising pandemic in Indian population with early onset than our western counterparts. Diabetes causes microvascular and macrovascular complications but microvascular complications like Diabetic Retinopathy, Diabetic Nephropathy, and diabetic Neuropathy are most common.

Diabetic dyslipidemia encompasses both quantitative and qualitative changes in plasma lipids and lipoproteins, characteristically observed in individuals with type-2 diabetes mellitus.

The study consisted of 130 patients, both inpatients and out patients from R L Jalappa hospital attached to Sri Devaraj Urs medical college who had met both inclusion and exclusion criteria.

In our study majority of patients were in the age group of 61-70 years and mean age at which patients had diabetes is 60.55±11.53 years, and mean age at which patients had microvascular complication was 63±10.51 years and mean age without microvascular complication was 57±11.82 which was similar to the study done by sasongko et al[141] which showed mean age with complications was 60 years and without complications was 58 years. In a study done by Patel ML et al[142], mean age was 62.5±11.2 years which was similar to the present study.

In our study out of 130 patients, 37(28.5%) were females and 93(71.5%) were males which showed male preponderance. In a study done by Lakhotia et al[143] out of 200 patients, 106(53%) were males and 94(47%) were females which also showed male preponderance.

In the present study mean duration of diabetes was 8.20±3.99 years. The mean age at which patients had microvascular complications was 9.22±3.77 years and patients without microvascular complication was 7.18±3.97 which is statistically significant comparatively. In

a study by Patel ML[142] et al, mean duration of diabetes was 11.5±7.6 years, in Sasongko et al[141] study it was 18 years and in a study by Lakhotia et al[143] mean duration of diabetes was 9.5±7.3 years. In this study it was lower than other studies which shows that duration of diabetes plays significant role in microvascular damage by prolonged hyperglycemia and advanced glycation end product accumulation over years and need for early screening for diabetic complications.

In this study all patients were on treatment for diabetes either on insulin, oral anti diabetics or both Out of 130 patients 29(22.3%) were on insulin, 78(60%) were on oral antidiabetic agents and 23(17.7%) on both. In a study by Patel ML et al[142],70% of patients were on OAD's.

In our study 56(56.9%) patients had history of hypertension, which was similar to a study done by Patel ML et al[142] which showed 39% patients had hypertension. In a study done by Toro et al[144], 66.4% patients had hypertension. Our study had lesser incidence of hypertension than the study done by Toro et al[144].

In this study mean Body Mass Index (BMI) kg/m^2 was 25.62±3.22 kg/m^2 and in patients with microvascular complication mean BMI was 25.74±3.25 kg/m^2 and in patients without microvascular complication was 23.51±2.80 kg/m^2 which was statistically significant (p value<0.001).

Table 29: Showing comparison of mean BMI in kg/m^2 in different studies.

Sl no	Study	Mean BMI kg/m^2
1	Chandini R et al[145]	25.16±3.9
2	Patel ML et al[142]	24.8±3.6
3	DCCT Trial[146]	25.6±3.2
4	Toro R et al	29.3±3.12
5	Present study	25.62±3.22

Our study correlates with study by Chandini R et al[145] and DCCT trial[146] which showed similar mean BMI.

In this present study 42(64.6%) patients with microvascular complications had diabetic retinopathy which is statistically significant (P value<0.001). Out of 42 patients 34(80.9%) had non proliferative diabetic retinopathy and 8(19.1%) patients had proliferative diabetic retinopathy which was similar to the study done by chandini R et al[145] which showed, out of 144 patients 66(45.8%) had diabetic retinopathy out of which 61(92.42%) had non proliferative diabetic retinopathy and 5(7.58%) patients had proliferative diabetic retinopathy which was similar to my study.

In the present study, patients with diabetic microvascular complication 50(78.1%) patients had diabetic nephropathy. All 50(78.1%) patients had microalbuminuria and 15(21.9%) patients had normoalbuminuria (n=65).

Table 30: Showing prevalence of diabetic nephropathy in patients with microvascular complication in various studies.

Sl no	Study	Prevalence
1	Chandini R et al[145] (n=144)	54.16%
2	Lakhotia et al[143] (n=200)	48%
3	Uniyal R[147] et al (n=64)	76.35%
4	Toro R et al[144] (n=217)	42.5%
5	Present study	78.1%

Our present study showed similar results as study done by Uniyal et al[147] where prevalence of diabetic nephropathy was 49(76.35%) n=64.

In the present study, out of 65 patients who had microvascular complications 32(49.2%) patients had diabetic neuropathy and out of which 9(28.1%) had mild neuropathy, 15(46.9%) had moderate neuropathy, 8(25.0%) had severe neuropathy.

Showing prevalence of diabetic neuropathy in other studies

Table 31: Showing prevalence of diabetic neuropathy.

Sl no	Study	Prevalence
1	Chandini R et al[145] (n=144)	37.5%
2	Lakhotia et al[144] (n=200)	41%
3	Subbalakshmi NK et al[148] (n=101)	52.4%
4	Rai NO et al[149] (n=1020	60.7%
5	Present study	49.2%

In the present study incidence of diabetic nephropathy was comparable with studies done by subbalakshmi et al[148] 53(52.4%) and lakhotia et al[143] 82(41%).

Overall incidence of microvascular complications n=65 in our study was 5(7.7%) patients had only Retinopathy, 13(20.0%) patients had only Nephropathy, and 5(7.7%) patients and had Neuropathy. 14(21.5%) patients had combination of both Retinopathy and Nephropathy, 5(7.7%) patients had combination of both Retinopathy and Neuropathy and 5(7.7%) patients had combination of both Nephropathy and Neuropathy and 18(27.7%) had all the three microvascular complication. (Retinopathy +Nephropathy +Neuropathy).

In our study patients mean SD of FBS was 152±69.35mg/dl and 149.15±41.59 mg/dl, with and without micro vascular complications respectively and Mean SD of PPBS was 270.62±69.35mg/dl and 251.57±70.24mg/dl with and without microvascular complications respectively. In a study done by Gupta RK et al mean SD of FBS was 182.6±71.6 mg/dl and 165±74.6 mg/dl, with and without micro vascular complications respectively and mean SD of

PPBS was 296±86.8 mg/dl and 264±67.76 mg/dl, with and without micro vascular complications respectively irrespective of type of microvascular complications which was comparable to this study.

In our study mean HbA1C in patients with microvascular complications was 8.75% and without micro vascular complications was 7.885 which was statistically significant.

In various studies mean HbA1C was

Table 32: Comparison of mean HbA1C levels in various studies.

Sl no	Study	HbA1C with microvascular complication	HbA1C without micro vascular complications
1	Rai ON et al[149]	8%	7.1%
2	Saongka et al[141]	8.0%	7.6%
3	Mallick et al[150]	10.34%	7.54%
4	Uniyal et al[147]	8.3%	6.8%
5	Lakhotia et al[143]	8.6%	-
6	Chandini et al[145]	8.01%	-
7	Present study	8.76%	7.88%

Our present study was comparable with studies done by Rai ON et al[149], Sasongka et al[141], chandini R et al[145] and was statistically significant with (p value 0.006).

In our study various traditional lipids were studied and mean values of total cholesterol was 170.88±53.03 mg/dl and 144.65±35.38 mg/dl, mean triglycerides was 160.65±55.12 mg/dl and 134.25±44.61 mg/dl, mean HDL was 35.28±12.18 mg/dl and 38.66±10.76 mg/dl, mean LDL level was 111.59±44.16 mg/dl and 100.72±36.19 mg/dl and mean VLDL was 43.18±54.36 mg/dl and 23.95±10.38 mg/dl was noted respectively in patients with micro vascular complications and without microvascular complications.

In various other studies

Mallick et al[150] found Triglyceride was 173.41 ± 79.52 and 238.15 ± 160.5 mg/dl, Total Cholesterol was 169.82 ± 43.79 mg/dl and 184.45 ± 43.95 mg/dl, HDL was 37.35 ± 11.57 and 31.85 ± 10.10 and LDL 97.79 ± 39.49 104.97 ± 41.56 respectively without microvascular and with microvascular complications.

Uniyal et al[147] found mean Total cholesterol was 167 ± 25 mg/dl and 170 ± 27 mg/dl, mean Triglycerides was 125 ± 22 mg/dl and 132 ± 29 mg/dl, Mean HDL was 42 ± 3.5 mg/dl and 41 ± 2.7 mg/dl, and Mean LDL was 98 ± 10.5 mg/dl and 99 ± 7.6 mg/dl respectively with or without microvascular complications

In our present study total cholesterol ($p<0.001$), triglycerides (p 0.003), and VLDL (p 0.006) were statistically significant and were higher in patients with microvascular complications than without microvascular complications patients whereas HDL was higher in patients without microvascular complication than with microvascular complication (p-0096) which was statistically significant and LDL (p - 0.127) statistically not significant but higher in patients with microvascular complications than patients without micro vascular complications.

Our study was comparable with study done by mallick et al[147] and dyslipidemia was observed in all diabetic patients with or without micro vascular complication with significant hypertriglyceridemia, reduced HDL levels, and an increased proportion of LDL and VLDL.

In our study in patients with diabetes mean lipoprotein (a) levels was 32.3±22.3mg/dl, mean apolipoprotein A1 was 123±41.96 mg/dl and mean Apolipoprotein level was 90.49±22.26 mg/dl which was statistically significant.

In other studies mean lipoprotein (a), mean apolipoprotein A1 and apolipoprotein B in mg/dl were

Table 33: Other studies showing mean lipoprotein (a), mean apolipoprotein A1 and apolipoprotein B in mg/dl with comparison of this study.

	Patel ML et al[142]	Toro R et al[144]	Present study
Lipoprotein (a)	55±10.5	33.30±12.3	32.3±22.3mg/dl
Apolipoprotein A1	140.5±32.8	133.3 ± 25.9	123±41.96
Apolipoprotein B	114.8±31.5	107.6 ± 20.9	90.49±22.26

Our present study does correlate with study done by Toro R et al and was statistically significant (P<0.001).

Levels of Lp (a), apolipoprotein A1, and apolipoprotein B in Diabetic retinopathy.

In our present study mean Lp (a), apolipoprotein A1 apolipoprotein B without diabetic retinopathy were 31.30±23.24 mg/dl, 120.98±45.62 mg/dl, and 91.90±22.72 mg/dl respectively and with diabetic retinopathy 34.13±20.84mg/dl, 127.87±34.81 mg/dl and

87.91±21.64 mg/dl respectively. The Apo B levels were higher in patient with diabetic retinopathy than patients without diabetic retinopathy but statistically not significant. The levels of Lp (a) and Apo A1 was lower in patients with retinopathy than without retinopathy patients and also statistically not significant.

In other studies the mean Lp (a) mg/dl levels with or without diabetic retinopathy was

Table 34: Comparison of Mean Lp (a) mg/dl levels with or without diabetic retinopathy.

Sl no	study	With diabetic retinopathy mg/dl	Without diabetic retinopathy mg/dl
1	Malaguarenara et al[151]	56.4	34.1
2	Lakhotia et al[143]	31.9	27.5
3	Chandini R et al[145]	26.4	19.3
4	Present study	34.13	31.30

Our study was comparable to the study done by Lakhotia et al[143] in which mean Lp (a) was higher in patients with retinopathy than in patients without retinopathy. In the present study and the study done by Lakhotia et al, the mean Lp (a) levels were not statistically significant.

Mean Apo A1 mg/dl levels with or without diabetic retinopathy was

Table 35: Comparison of Mean Apo A1 mg/dl levels with or without diabetic retinopathy.

Sl no	Study	With diabetic retinopathy mg/dl	Without diabetic retinopathy mg/dl
1	Sasongko et al	150	140
2	Malaguarenara et al	154	147
3	Present study	127.87	120.98

Our study was comparable with the study done by Sasongko et al[141] in which mean Apo A1 levels were higher in patients with retinopathy than in patients without retinopathy but is not statistically significant (p-0.531).

Mean Apo B mg/dl levels with or without diabetic retinopathy was

Table 36: Comparison of Mean Apo B mg/dl levels with or without diabetic retinopathy.

Sl no	Study	With diabetic retinopathy mg/dl	Without diabetic retinopathy mg/dl
1	Sasongko et al	90	80
2	Malaguarenara et al	108	106
3	Present study	91.90	87.91

In our study the mean Apo B levels were comparable with the studies done by Sasongko et al[141] in which mean Apo B levels were higher in patients with diabetic retinopathy than in patients without diabetic retinopathy but was not statistically significant in our study (p-0.494).

Levels of lipoprotein (a), apolipoprotein A1, and apolipoprotein B in Diabetic nephropathy:

In our present study mean Lp (a), apolipoprotein A1 apolipoprotein B with diabetic nephropathy were 36.11±23.34mg/dl,125.68±41.96mg/dl and 92.14±22.82 mg/dl respectively and without diabetic nephropathy was 19.61±11.90mg/dl, 115.87±42.52 mg/dl and 85.00±20.00 mg/dl respectively. The Lp(a) levels were higher in patient with diabetic nephropathy than patients without diabetic nephropathy and was statistically significant. The levels of Apo A1 and Apo B was higher in patients with nephropathy than without nephropathy patients but was not statistically significant.

In other studies, the mean Lp (a) mg/dl levels with and without nephropathy was

Table 37: Comparison of Mean Lp (a) mg/dl levels with or without diabetic nephropathy

Sl no	Study	Nephropathy present in mg/dl	Nephropathy absent in mg/dl
1	Lakhotia et al[143]	35.7	24.1
2	Toro R et al[144]	27.0	17.6
3	Patel ML et al[142]	42.5	35.5
4	Umashankar et al[153]	35.7	24.1
5	Present study	36.11	19.61

Lp (a) was increased in Diabetic Nephropathy compared to Diabetes without Nephropathy. But the increase in the level of Lp (a) in Nephropathy patients was significant in the above said series even though the difference was statistically significant (P<0.011).

Mean Apo A1 mg/dl levels with and without nephropathy was

Table 38: Comparison of Mean Apo A1 mg/dl levels with or without diabetic nephropathy.

Sl no	study	Nephropathy present in mg/dl	Nephropathy absent in mg/dl
1	Toro R et al[144]	133.7	134
2	Patel ML et al[142]	140.2	137
3	Present study	125.68	115.87

Apo A1 was increased in Diabetic Nephropathy compared to Diabetes without Nephropathy. But the increase in the level of Apo A1 in Nephropathy patients was not significant in the above mentioned series and difference was not statistically significant (P-0.431).

Mean Apo b mg/dl levels with and without nephropathy was

Table 39: Comparison of Mean Apo B mg/dl levels with or without diabetic nephropathy

Sl no	study	Nephropathy present in mg/dl	Nephropathy absent in mg/dl
1	Toro R et al[144]	111.5	106.8
2	Patel ML et al[142]	118.4	106.8
3	Present study	92.14	85.00

Apo B was increased in Diabetic Nephropathy compared to Diabetes without Nephropathy. But the increase in the level of Apo B in Nephropathy patients was not significant in the above mentioned series and difference was not statistically significant (P-0.279).

Levels of lipoprotein (a), apolipoprotein A1, and apolipoprotein B in Diabetic neuropathy.

In the present study,the mean Lp (a), apolipoprotein A1 apolipoprotein B with diabetic neuropathy was 33.16±21.19 mg/dl, 119.94±44.32 mg/dl and 87.55±18.11mg/dl respectively and without diabetic neuropathy was 31.46±23.62 mg/dl, 127.00±39.76 mg/dl and 93.53±25.79 mg/dl respectively. The mean Lp(a), Apo A1, and Apo B levels was higher in patients without diabetic neuropathy than patients with diabetic neuropathy and also the levels was not statistically significant.

CONCLUSION

Serum Dyslipidemia is much more common in type-2 diabetes with microvascular complications compared to type-2 diabetes without microvascular complications.

Serum Dyslipidemia (hypertriglyceridemia, LDL) is one important risk factor for microvascular complications and Lp (a), Apo A1 and Apo B are also novel emerging lipid risk factor.

Increased Lp (a), Apo A1, and Apo B and Triglycerides, LDL may be the reason for increased prevalence of microvascular complications.

Poor control of DM is the lone significant predictor of elevated Lipoprotein (a), Apo A1 and Apo B followed by duration of Diabetic >10 years.

Lp (a), Apo A1 levels were higher in patients with diabetic retinopathy but not statistically significant. Apo B levels were elevated in diabetic retinopathy but not statistically significant. Lp (a) were significantly raised and statistically significant in diabetic nephropathy patients. Apo A1 and Apo B were elevated but not statistically significant.

Lp (a), Apo A1 and Apo B levels were elevated in patients with neuropathy but was not statistically significant.

From this study we can opine that most patients in the group with microvascular complication, diabetic retinopathy followed by diabetic nephropathy followed by diabetic neuropathy were the common microvascular complication.

Most of the patients had more than one microvascular complication in increasing order and the increase in the total cholesterol, triglycerides, LDL, VLDL and Lp (a), Apo A1 and Apo B and decrease in the HDL in type-2 diabetes with retinopathy, nephropathy, neuropathy are

probably because of the effect of increased duration and poor control of diabetes on lipid parameters and novel extended lipids and the consequent microvascular complications.

From this study we came to the inference that regular lipid parameters were elevated as from previous studies and novel extended lipid parameters Lp (a), Apo A1 and Apo B may be used in association or independently as biomarkers for diabetic retinopathy, diabetic nephropathy, and diabetic neuropathy.

Further studies are needed to correlate and associate diabetic neuropathy and novel extended lipid parameters.

SUMMARY

Type 2 Diabetes Mellitus is one of the common medical illness and serious metabolic disorder which is rapidly raising pandemic in Indian population with early onset than our western counterparts. Diabetes causes microvascular and macrovascular complications but microvascular complications like Diabetic Retinopathy, Diabetic Nephropathy, and diabetic Neuropathy are most common.

Establishment of extended novel lipid abnormalities Lp (a), Apo A1 and Apo B and their relationship with increased microvascular complications in type-2diabetes with Diabetic retinopathy, nephropathy and neuropathy was the main theme of this hospital based comparative cross sectional study.

Review of literature regarding type-2diabetes with microvascular complications and without microvascular was done. Present study was viewed in comparison with other national and international studies.

The study group consisted of 130 cases, 65 in type-2 diabetes with microvascular complication group and 65 in type-2 diabetes without microvascular complications group. Average age in type-2 diabetes with microvascular complications group was 63.51±10.51 years and in type-2 diabetes without microvascular complications group was 57.60±11.82 years. They were matched for sex, BMI, diabetic control, and hypertension control.

Serum regular lipid profile and novel extended lipid parameters Lp (a), Apo A1 and Apo B of 65 type-2diabetes with microvascular complications was studied and compared with those of type-2 diabetes without microvascular complications.

The salient features of this study are-

1. The prevalence of diabetic nephropathy was higher than retinopathy and neuropathy
2. The patients with diabetes with microvascular complications had higher FBS, PBBS and HbA1C levels compared to patients without microvascular complications. This indicates that patients with microvascular complications had poor glycemic control compared to patients without microvascular complications.
3. The mean values of lipid fractions like Lp (a), Apo A1, Apo B, serum cholesterol, triglycerides, LDL and VLDL were higher in type-2diabetes with microvascular complications when compared to type-2 diabetes without microvascular complications.
4. Abnormal Lipoprotein (a), Apo A1 and Apo B levels was noted in 26.9%, 38.5%, and 50.8% of patients with diabetic microvascular group.
5. Patients with diabetic retinopathy had higher levels of Apo A1 than other extended novel lipid parameters. Lp (a) levels were higher in patients with diabetic nephropathy compared to other novel lipid parameters $p<0.011$. Patients with diabetic neuropathy had higher levels of novel lipid parameters but not statistically significant.
6. As the duration of Diabetes increases the proportion of abnormal Lipoprotein (a), Apo A1 and Apo B also increases as the patients with microvascular complications had higher duration of diabetes than patients without microvascular complications. P-0.003.
7. The mean values of lipid fractions like Total cholesterol, triglycerides, LDL, VLDL, Lp (a), Apo A1, Apo B were higher in patients with microvascular complications compared to patients without microvascular complications.
8. The most abnormal Dyslipidemia seen in the diabetic microvascular patients group is elevated Triglyceride, VLDL and Lp (a) followed by Apo A1and Apo B.

9. Diabetic neuropathy patients need further studies to find out mechanism and association with novel extended lipid parameters Lp (a), Apo A1, Apo B.

From this study we can infer that novel extended lipid parameters may be used as the new biomarkers for diabetic microvascular complications.

BIBLIOGRAPHY

1. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 1997 ;20:1183–1197.
2. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Follow-up report on the diagnosis of diabetes mellitus. Diabetes Care 2003;26:3160–3167.
3. Fowler MJ. Microvascular and Macrovascular complications of diabetes. Clinical Diabetes 2008;26(2):77-82.
4. Mooradian AD. Dyslipidaemia in type 2 diabetes mellitus. Nature Clinical Practice Endocrinology & Metabolism 2009;5:150-159.
5. Singla S, Kaur K, Kaur H. Lipoprotein (a) in type 2 diabetes mellitus: Relation to LDL: HDL ratio and glycaemic control. Int J Diabetes Dev Ctries 2009;29:80-84.
6. Chang Y-C, Wu W-C. Dyslipidaemia and Diabetic Retinopathy. Rev diabet stud 2013;10:121-132.
7. Thomas HH. Diabetic Nephropathy in the kidney, 4th edition,Brenner and rector(Edtrs) Philadephia,W.B.Saunders 1991:1695-1719.
8. Leo P. Carcl, Donald Bornett, Rachmid Levine. The History of Diabetes in Joslin's Diabetes Mellitus, E Ronald Kahn, Gordon C weir(Edtrs),New Delhi,BI Waverly pvt ltd: 1-13.
9. Kalantzis G, Micheal A, Effie PR. Diabetic Retinopathy: An Historical assessment. Hormones 2006;5(1):72-75.
10. Bouchard M. Sur la perte des reflexes tendineux dans le diabete sucre. Progress Med 1884;12:819.

11. Pavy FW. Introductory address to discussion of the clinical aspect of glycosuria. Lancet 1885;2:1085.
12. Alvin C. Powers; Diabetes mellitus, Harrison's principles of internal medicine vol 2, 16th edi. Edited by Braunwald p 2152.
13. King H, Aubert RE, Hermal.WH. Global burden of diabetes 1995-2025; prevalence, numerical estimates and projection. Diabetes care 1998; 21:1414-31.
14. World Health Organization, Global Report on Diabetes. Geneva, 2016.
15. Wild S, Roglic G, Green A, Sicree R, King H (2004). "Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030". Diabetes Care 2015;27(5):1047–53.
16. Agardh E et al. Type 2 diabetes incidence and socio-economic position: a systematic review and meta-analysis. International Journal of Epidemiology 2011;40(3):804-818.
17. Classification and Diagnosis of Diabetes. American diabetic association. Diabetes Care 2016;39(1):S13–S22.
18. American diabetes Association: screening for type 2 diabetes, 2004.
19. Chang SA. Smoking and Type 2 Diabetes Mellitus. Diabetes Metab J 2012;36:399-403.
20. Soo-Jeong K, Dai-Jin K. Alcoholism and Diabetes Mellitus. Diabetes Metab J 2012;36:108-115.
21. Powers AC. Diabetes Mellitus: Complications. Harrison's Principles of Internal Medicine, 19th edition. Kasper, Fauci, Hauser, Longo et al (Edtrs) 2015 McGraw-Hills Education. P-2422
22. Joanna M T, Kirti K, Mohit C, Eva MK,Rakesh C. Pathophysiology of Diabetic Retinopathy. Hindawi Publishing Corporation. Ophthalmology 2013, 13 pages.

23. Donald SF, Lloyd A, Thomas WG et al. Diabetic Retinopathy. Diabetes Care 2003;26(1):s99-s102.
24. Grading diabetic retinopathy from stereoscopic colour fundus photographs--an extension of the modified Airlie House classification. ETDRS report number 10. Early Treatment Diabetic Retinopathy Study Research Group. Ophthalmology 1991;98:786.
25. Leese GP, Morris AD, Olson J. A national retinal screening programme for diabetes in Scotland. Diabet Med 2003;20:962.
26. Jeganathan VS, Wang JJ, Wong TY. Ocular associations of diabetes other than diabetic retinopathy. Diabetes Care 2008;31(9):1905-12.
27. Gabbay KH. Hyperglycemia, polyol metabolism, and complications of diabetes mellitus. Annual Review of Medicine 1975;26:521–536.
28. Kinoshita JH. A thirty year journey in the polyol pathway. Experimental Eye Research 1990;50(6):567–573.
29. Lassègueand B, Clempus E. VascularNAD(P)Hoxidases: specific features, expression and regulation. AmericanJournal of Physiology 2003;285(2):277-297.
30. Frank RN, Keirn RJ, Kennedy A, Frank KW. Galactose-induced retinal capillary basement membrane thickening: prevention by sorbinil. Investigative Ophthalmology and Visual Science 1983;24(11):1519–1524.
31. Koya D, King GL. Protein kinase C activation and the development of diabetic complications. Diabetes 1998;47(6);859–866.
32. Aiello LP, Clermont A, Arora V, Davis MD, Sheetz MJ, Bursell SE. Inhibition of PKC$\beta\beta$by oral administration of ruboxistaurin is well tolerated and ameliorates diabetes induced retinal hemodynamic abnormalities in patients. Investigative Ophthalmology and Visual Science 2006;47(1):86–92.

33. Grant MB, Mames RN, Fitzgeraldetal C. Efficacy of octreotide in the therapy of severe nonproliferative and early proliferative diabetic retinopathy: a randomized controlled study. Diabetes Care 2000;23(40):504–509.
34. Hueber A, Wiedemann P, Esser P, Heimann K. Basic fbroblast growth factor mRNA, bFgF peptide and FGF receptor in epiretinal membranes of intraocular proliferative disorders (PVR and PDR). International Ophthalmology 1996;20(6):345–350.
35. Simó R, Hernández C. Intravitreous anti-VEGF for diabetic retinopathy: Hopes and fears for a new therapeutic strategy. Diabetologia 2008;51(9):1574–1580.
36. G. M. Comer and T. A. Ciulla, "Pharmacotherapy for diabetic retinopathy,"CurrentOpinioninOphthalmology,vol.15,no.6, pp.508–518,2004.
37. Takahashiand H, Shibuya M.Vascular endothelial growth factor (VEGF)/VEGF receptor system and its role under physiological and pathological conditions. Clinical Science 2005;109(3):227–241.
38. Otani A, Takagi H, Oh H et al. Angiotensin II-stimulated vascular endothelial growth factor expression in bovine retinal pericytes. Investigative Ophthalmology and Visual Science 2000;41(5):1192–1199.
39. Hammes HP, Martin S, Federlin K, Geisen K, Brownlee M. Amino guanidine treatment inhibits the development of experimental diabetic retinopathy. Proceedings of the National Academy of Sciences of the United States of America 1991;88(24):11555–11558.
40. Ulrich P, Cerami A. Protein glycation, diabetes and aging. Recent Progress in Hormone Research 2001;56:1–21.
41. Zong H, Ward M, Stitt AW. AGEs, RAGE, and diabetic retinopathy. Current Diabetes Reports 2011;11(4):244–252.

42. Baynes JW. Role of oxidative stress in development of complications in diabetes. Diabetes 1991;40(4):405–412.
43. El-Remessy AB, Bartoli M, Platt DH, Fulton D, Caldwell R. Oxidative stress inactivates VEGF survival signaling in retinal endothelial cells via PI 3-kinase tyrosine nitration. Journal of Cell Science 2005;118(1):243–252.
44. Enden MKV, Nyengaard JR, Ostrow E, Burgan JH, Williamson JR. Elevated glucose levels increase retinal glycolysis and sorbitol pathway metabolism: implications for diabetic retinopathy. Investigative Ophthalmology and Visual Science 1995;36(8):1675–1685.
45. Barber AJ, Gardner W, Abcouwer SF.The signifcance of vascular and neural apoptosis to the pathology of diabetic retinopathy. Investigative Ophthalmology and Visual Science 2011;52(2):1156–1163.
46. Park SH, Park JW, Park SJ et al. Apoptotic death of photoreceptors in the streptozotocin-induced diabetic rat retina. Diabetologia 2003;46(9):1260–1268.
47. Lim LS, Wong TY. Lipids and diabetic retinopathy. Expert Opin Biol Ther 2012;12(1):93-105.
48. Huang Q, Yuan Y. Interaction of PKC and NOS in signal transduction of microvascular hyperpermeability. Am J Physiol 1997;273:H2442-H2451.
49. Stitt AW. AGEs and diabetic retinopathy. Invest Ophthalmol Vis Sci 2010;51(10):4867-4874.
50. Gu X, Meer SG, Miyagi M, Rayborn ME, Hollyfield JG, Crabb JW, Salomon RG. Carboxyethylpyrrole protein adducts and autoantibodies, biomarkers for age-related macular degeneration. J Biol Chem 2003;278(43):42027-42035.
51. Zong H, Ward M, Stitt AW. AGEs, RAGE, and diabetic retinopathy. Current Diabetes Reports 2011;11(4):244-252.

52. Hill SA, McQueen MJ. Reverse cholesterol transport - a review of the process and its clinical implications. Clin Biochem 1997;30(7):517-525.
53. Tserentsoodol N, Gordiyenko NV, Pascual I, Lee JW, Fliesler SJ, Rodriguez IR. Intraretinal lipid transport is dependent on high density lipoprotein-like particles and class B scavenger receptors. Mol Vis 2006. 12:1319-1333.
54. Robbesyn F, Auge N, Vindis C, Cantero AV, Barbaras R, Negre-Salvayre A, Salvayre R. High-density lipoproteins prevent the oxidized low-density lipoproteininduced epidermal growth factor receptor activation and subsequent matrix metalloproteinase-2 upregulation. Arterioscler Thromb Vasc Biol 2005;25(8):1206-1212.
55. Marcovina S, Packard CJ. Measurement and meaning of apolipoprotein AI and apolipoprotein B plasma levels. J Intern Med 2006;259(5):437-446.
56. McQueen MJ, Hawken S, Wang X, Ounpuu S, Sniderman A, Probstfield J, Steyn K, Sanderson JE, Hasani M, Volkova E, et al. Lipids, lipoproteins, and apolipoproteins as risk markers of myocardial infarction in 52 countries (the INTERHEART study): a case-control study. Lancet 2008;372(9634):224-233.
57. Sarita Bajaj, DM (Endocrinology AIIMS), Associate Prof Medicine, MLN Medical College, Allahabad.
58. V Kannan (DM Nephrology). Renal diseases in diabetes mellitus: Type-2 Diabetes Mellitus: 2004:172-194
59. Viberti GC et al. Protienuria in diabetes mellitus: role of spontaneous and experimental variation of glycemia". Kidney Int 1982 May; 21(5): 714-720.
60. Parving HH, Oxenball B, Svendsen PA et al. Early edetection of patients at risk of developing diabetic nephropathy: a longitudinal study of urinary albumin excretion. Acta endocrinal(Copenh) 1982; 100:550-5.

61. Viberti GC, Hill Rd, Jarrett RJ,et al. Microalbuminuria as a predictor of clinical nephropathy in insulin dependent diabetes mellitus. Lancet, 1982; 1:1430-1432.
62. Mogensen CE, Christensen CK. Predicting diabetic nephropathy in insulin dependent patients. N Engl J Med 1984; 311: 89-93.
63. Damsgaard EM, Froland A. Eight to nine year mortality in known noninsulin dependent diabetes and controls. Kidney Int 1992;41:731-735.
64. Damsgaard EM, Froland A. et al. Microalbuminuria as a predictor of increased mortality in elderly people. BMJ 1990; 300: 297-300.
65. Schmitz A, Vaeth M. Microalbuminuria: A major risk factor in noninsulin dependent diabetes. A 10-year follow-up study of 503 patients. Diabe Med 1988;5:126-134.
66. Deckert T, Feldt-Rasmussan B, Jensen T et al. Albuminuria reflects wide spread vascular damage: The steno hypothesis. Diabetologia 1989;32:219-226.
67. Consensus development Conference on the diagnosis of Coronary Heart Disease in people with Diabetes. Diabetes care 1998;21:1551-68.
68. Jotideb Mukhopadhyay. Clinical implications of Microalbuminuria, Chapter 65 in API's Medicine Update. Venkatraman S. 2004;14: 401-404.
69. Tetsuya B, Chieko T, Yasuhiko I. Definition of Microalbuminuria in first morning and Random Spot Urine in diabetic Patients. Diabetes care 2004;27(7): 1838-1839.
70. Keane WF, Eknoyan G. Protienuria albuminuria risk, assessment, detection, elimination (PARADE): A position paper of the National Kidney foundation. Am J Kidney Dis1999; 33: 1004-1010.
71. Albuminuria and Vascular Damage –The Vicious Twins Editorial by Eberhand Ritz, M D. N Engl J Med; 348:123: June 5 2003-2349-51.

72. Astin MA,King M-C, Vranizan KM, Krauss RM. Atherogenic lipoprotein phenotype a proposed genetic marker for coronary heart disease risk Circulation 1990; 82: 495-509.
73. Astin MA, Edwards KL,Small, dence low density lipoproteins, the insulin resistance syndrome and noninsulin dependent diabetes. Curr Opin Lipidol 1996; 7:167-71.
74. Expert Panel on Detection and Treatment of High Blood Cholesterol in Adults, National Cholesterol Education Program, Second report of the Expert Panel on Detection Evaluation and Treatment of High Blood Cholesterol (Adult Treatment Panel 11). Circulation 1994; 89:1333-445.
75. Messent JW, Elliott TG, Hill RD, Jarret RJ, Viberti GC. Prognostic significance of microalbuminuria in insulin dependent diabetes mellitus: A 23 year follow-up study. Kidney Int 1992;41:836-9.
76. Stamler J, Vaccaro O, Neaton J, for the Multiple Risk Factor Intervention Trial Research Group. Diabetes, other risk factors, and 12-year cardiovascular mortality for men screened in the multiple risk factor intervention trial. Diabetes Care 1993;16:434.
77. Uusitupa MJ, Niskanen LK, Siitonen O, Voutilainen, E, Pyorala K. Ten year cardiovascular mortality in relation to risk factors and abnormalities in lipoprotein composition in Type-2 diabetic and non-diabetic subjects. Diabetologia 1993;36:1175-84.
78. Csaszar A, Dieplinger H, Sandholzer C, Karadi J, Juhast E, Drevel H. Plasma lipoprotein(a) concentration and phenotypes in diabetes mellitus. Diabetologia 1993; 36:47-51.
79. Christoph Hasslacher. Diabetic Nephropathy: 2001
80. Schlondroff D.

81. Cellular mechanisms of lipid injury in the glomerulus, Am J Kidney Dis 1993; 22: 279-85.
82. Samulsson O, Aurell M, Knight-Gibson C, Alaupovic P, Attman P-O. Apolipoprotein-B-containing lipoproteins and the progression of renal insufficiency. Nephron 1993;63:279-85.
83. American Diabetes Association, American Academy of neurology report andrecomendations of the San Antonio Conference on Diabetic Neuropathy(consensus statement). Diabetic Care 1988;11:592-597.
84. Dyck PJ, Kratz KM, Karnes JL, et al. The prevalence by staged seviarity of various types of diabetic neuropathy, retinopathy, nephropathy in a population based cohort: The Rochester Diabetic Neuropathy study. Neurology 1993;43:817.
85. Pradeepa R, Rema M, Vignesh J, Deepa R, Deepa M, Mohan V. Prevalence and risk factors for diabetic neuropathy in an urban south Indian population: the Chennai Urban Rural Epidemiology Study (CURES-55). Diabetic Medicine 2008;25(4):407-412.
86. Leydon E Beitrage Zur Klinic des Diabetes Mellitus. Wein Med Wochenchr 1893;43:926.
87. Dyck J, Thomas PK. Diseases of the peripheral nervous system 4th edition 2005:1953.
88. Vinik AI. Diabetic neuropathy: pathogenesis and therapy. Am J Med 1999;107:17-26.
89. Yagishashi S, Mizukani H, Sugimoto K. Mechanism of Diabetic neuropathy: Where are we now and Where to go. Journal of Diabetic Investigation 2011;2(1):18-32.
90. Dyck J. Detection, characterization and staging of polyneutopathy assesd in Diabetes. Muscle Nerve 1988;11:21-32.

91. Vincent AM, Lucy MH, Pop-Busui R, Feldmana EL. Hyperlipidemia: A New Therapeutic Target for Diabetic Neuropathy. J Peripher Nerv Syst 2009;14(4):257-267.
92. Peter A. Mayer, Lipids of Physiologic Significance. Harper's Biochemistry. 24th edition – Lange Medical Publications, Page No. 146-155.
93. Chatterjia MN, Rana S. Chemistry of Lipids in Text Book of Medical Biochemistry, 7th edition, New Delhi, Jaypee Brothers 2008;45.
94. Review of Medical Physiology – 19th Edition – William F. Ganong – Lange Medical Publications – Chapter 17 – Energy Balance, Metabolism and Nutrition. Page No.288-294.
95. John Betteridge. Lipid disorders in diabetes; Diabetes clinical management. 1st Edition, 1990. Edited by Robert B Tatttersall. Pg. 333-342.
96. Robert W. Mahley, Karl H. Weisgraber. Disorder of lipid metabolism In William's Textbook of Endocrinology. 10th ed WB Saunders (Philadelphia) 2003:1643-56
97. Daniel JR, Helen HH. Disorders of Lipoprotein Metabolism, Harrison's Principles of Internal Medicine Vol. 2, 19th edition, 2286-2296.
98. Steinberg D. Lipoproteins and the pathogenesis of atherosclerosis. Circulation 1987;76: 508-514.
99. Steinberg D, Carew TE. Beyond cholesterol: modifications of low-density lipoprotein that increases atherogenicity. N Engl J Med 1989;320:915-924.
100. Witztum JL. The oxidation hypothesis of atherosclerosis. Lancet 1994;793-795.
101. Rye K-A, Clay MA. Remodelling of high-density lipoproteins by plasma factors. Atherosclerosis 1999;145:227-238.
102. Berg K. A new serum type system in man, the LP system. Acta Patho Microbial Scand 1963;59:369.

103. Ehnolm C, Garoff. Purification and quantification of the human plasma lipoprotein carrying the Lp (a) antigen. Biochem Biophys Acta 1975;239: 431.

104. Dahlen G. The Lp (a) and coronary artery disease. Acta Med Scand (Suppl) 1974; 570: 571.

105. Bernadine H. Lp (a) joins other serum cholesterol lipoproteins as risk determinant. JAMA 1989;261:2013-14.

106. Utermann G. The mysteries of lipoprotein (a). Science 1989;246:904-910.

107. Scanu AM, Fless GM. Lp (a): Heterogeneity and biological relevance. J Clin Invest 1990;85;1709-1715.

108. Dahlen G, Guyton JR. Association of the levels of Lp (a), plasma lipids and other lipoproteins with coronary heart disease documented by angiography. Circulation 1986; 74:758-765.

109. Genest J, Jenner JL. Prevalence of Lp (a) in excess in coronary heart disease. Am J Cardiol 1991; 67:1039-1045.

110. Mahley RM, Thomas L, Rall SC, Weisgraber KH. Plasma lipoproteins: apolipoprotein structure and function. Journal of Lipid Research 1984;25:1277-1294.

111. Fidge NH. Nestel PJ, Ishikawa T, Reardon M, Billington T. Turnover of apoproteins A-I and A-I1 of high density lipoprotein and the relationship to other lipoproteins in normal and hyperlipidemic individuals. Metabolism 1984;29: 643-653.

112. Zong NC, Li H, Lam MP, Jimenez RC, Kim CS, Deng N, Kim AK, Choi JH, Zelaya I et al. Integration of cardiac proteome biology and medicine by a specialized knowledgebase. Circulation Research 2013;113(9): 1043–53.

113. Su Q, Tsai J, Xu E, Qiu W, Bereczki E, Santha M, Adeli K. Apolipoprotein B100 acts as a molecular link between lipid-induced endoplasmic reticulum stress and hepatic insulin resistance. Hepatology 2011;50(1):77–84.

114. Packard CJ, Shepherd J. Lipoprotein Heterogeneity and Apolipoprotein B Metabolism. Arteriosclerosis, Thrombosis, and Vascular Biology 1997;17:3542-3556.

115. Gaw A, Packard CJ, Lindsay GM, Griffin BA, Caslake MJ, Lorimer AR, Shepherd J. Overproduction of small very low density lipoproteins (Sf 20–60) in moderate hypercholesterolemia: Relationship between apolipoprotein B kinetics and plasma lipoproteins. J. Lipid Res 1995;36:158–171.

116. Boren J, Rustaeus S, Olofsson SO. Studies on the assembly of apolipoprotein B-100 and B-48 containing very low density lipoproteins in McA-RH7777 cells. J. Biol. Chem 1994;269:25879–25888.

117. Russell Ross and John A. Glomet. The Pathogenesis of Atherosclerosis (First of Two Parts). N Engl J Med 1976;295: 369-375.

118. Scott PJ, Hurley PJ. The distribution of radio-iodinated serum albumin and low- density lipoprotein in tissues and the arterial wall. Atherosclerosis 1970;11:77-103.

119. Sterry HC. The sequence of cell and matrix changes in atherosclerotic lesions of coronary arteries in the first forty years. Eur Heart J 1990;11(Suppl E):3-19.

120. Ross R. Atherosclerosis: An inflammatory disease. NEJM 1990;340:115-126.

121. John Betteridge. Lipid disorders in diabetes; Diabetes clinical management. 1st edition, 1990. Edited by Robert B Tatttersall. P 333-342.

122. Alan C, Steven H, Diabetes, Lipids and atherosclerosis; Endocrinology vol 1, 14th edition. Edited by Leslie J. Degroot.p 941-950.

123. Haffner SM: Management of dyslipidemia in adults with diabetes. Diabetes Care 1998;21:160-178.

124. Barrett-Connor E, Grundy SM. Holdbrook MJ: Plasma lipids and diabetes mellitus in an adult community. Am. J Epidemiol 1982;15:657-663.

125. Wilson PW, Kannel WB, and Anderson KM: Lipids, glucose intolerance and vascular disease: Framingham study. Monogr Atheroscter 1985;13:11.

126. Abbate SL. Brunzell JD: Pathophysiology of hyperlipidemia in diabetes mellitus. J cardiovasc Pharmacol 1990;16(Suppl):1-7.

127. Nikkila EA. High density lipoproteins in diabetes. Diabetes 1981;30:82-87.

128. Mohan et al. Lipid India 1996.

129. Dyslipidemia Management in adults with Diabetes. Diabetes care 2004;27(1):68-70.

130. Marcovina S, Packard CJ. Measurement and meaning of apolipoprotein AI and apolipoprotein B plasma levels. Journal of Internal Medicine 2006;259: 437–44.

131. Barter P, Kastelein J, Nunn A, Hobbs R. Future Forum Editorial Board. High density lipoproteins (HDL) and atherosclerosis; the unanswered questions. Atherosclerosis 2003;168: 195–200.

132. Chan DC, Bennet PHR, Watts GF. Dyslipidemia in visceral obesity: mechanisms, implications and therapy. Am J Cardiovasc Drugs 2004;4:227–46.

133. Matthan NR, Jalbert SM, Lamon-Fava S et al. TRL, IDL and LDL apolipoprotein B and HDL apolipoprotein AI kinetics as a function of age and menopausal status. Arterioscler Thromb Vasc Biol 2005;25:1691–6.

134. Packard CJ, Demant T, Stewart JP et al. Apolipoprotein B metabolism and the distribution of VLDL and LDL subfractions. J Lipid Res 2000;41:205–18.

135. Roeschlau P, Bernt E and Gruber WA. Clin Biochem. 1974;12(226):13-14.

136. Trinder P. Ann Clin Biochem 1969;6:24-7.

137. Gordon T, Castelli WP, Hjortland MC et al. Am. J. Med. 1977;62:707-714.

138. Alan TR, Nader R, Russell GW. Lipids, lipoproteins, apoproteins and other cardiovascular risk factors. In, Brutis CA, Ashwood ER, Burns DE. Teitz text book of

clinical chemistry and molecular diagnosis. 5th edition published by Elsevier,a division of Reed Elsevier India private Ltd, 2012;772.

139. Roeters VL. On treatment Apolipoprotein and Recurrent CAD events; Arterioscler Thromb Vasc Biol. 2000;4:2408-13.
140. Talmund. Apo B and TG levels predict CHD risk, Arterioscler Thromb Vasc Biol. 2002;5:1918–22.
141. Sasongko MB, Wong TY, Nguyen TT, Kawasaki R, Jenkins A, Wang JJ. Serum Apolipoprotein AI and B Are Stronger Biomarkers of Diabetic Retinopathy Than Traditional Lipid. Diabetes Care 2011;34:474–479.
142. Patel ML, Sachan R, Gupta KK. Correlation of plasma apolipoprotein and lipid profiles with different stages of type 2 diabetic nephropathy-A hospital based study in north India population. International journal of scientific and research publication 2012;2:234-238.
143. 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.
144. Toro R, Segura E, Alipio M et al. Relationship between lipoprotein (a) and micro/macro complications in type 2 diabetes mellitus: a forgotten target. Journal of Geriatric Cardiology 2015;12:93−99.
145. Chandni R, Ramamoorthy KP. Lipoprotein (a) in type 2 diabetic subjects and its relationship to diabetic microvascular complications. World J Diabetes 2012;3:105–109.
146. Nathan DM. The Diabetes Control & Complications Trial/Epidemiology of Diabetes intervention & complications study at 30 years; overview. Diabetes Care 2014;37:9-16.
147. Uniyal R, AHUJA R, Ojha, Sharma S, Uniyal D. Correlation of ApoB/ApoA1 with diabetic nephropathy. Brunei Int Med J. 2012;8(4):179-184

148. Subbalakshmi NK, Sathyanarayana RKN, Adhikari PMR, Sheila RP. Influence of dyslipidemia on somatic neuropathy in type 2 diabetes mellitus. Nitte University Journal of Health Science 2013;3(3):25-30.

149. Rai ON, Mishra V, Chandra R, Saxena SK, Mangal BD. Diabetic Peripheral Neuropathy And Its Metabolic Determinants In A North Indian Population. NJIRM 2016;7(2):1-4.

150. Mallick AJ, Maradi R, Joshi VR, Bhat G. A study on apo b100/apo a-i ratio in uncontrolled type 2 diabetes mellitus. International Journal of Applied Biology and Pharmaceutical Technology 2011;2(1):379-384.

151. Malaguarnera G, Gagliano C, Bucolo C, Vacante M, Salomone S, Malaguarnera M, Leonardi GD. Lipoprotein(a) Serum Levels in Diabetic Patients with Retinopathy. Bio Med Research International 2013:1-5.

152. Prakash G, Agrawal R, Satsangi SK, Prakash S. Comparison of Serum Apolipoproteins and Traditional Lipids in Eyes with Diabetic Retinopathy in Indian Population: A Case Series. Middle East African Journal of Ophthalmolog 2016;23(2):1-5.

153. Umashankar M, Ray G, Rina M, Das PK, Shah D. Lipoprotein abnormalities in type 2 diabetes with microalbuminuria. J Assoc Phy India 2003;31:1167.

ANNEXURES

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH

TAMAKA, KOLAR-563101

INFORMED CONSENT FORM FOR CLINICAL STUDIES

Informed Consent form for **"A COMPARATIVE STUDY OF ASSOCIATION OF EXTENDED LIPID PROFILE WITH MICROVASCULAR COMPLICATIONS IN TYPE 2 DIABETES MELLITUS."**

This Informed Consent Form is for men and women who attend the outpatient and inpatient at R.L.Jalappa Hospital and who we are inviting to participate in research on **Type Diabetes Mellitus.**

The title of our research project is **"A COMPARATIVE STUDY OF ASSOCIATION OF EXTENDED LIPID PROFILE WITH MICROVASCULAR COMPLICATIONS IN TYPE 2 DIABETES MELLITUS."**

The study will be conducted by Dr. Likitesh A B under the guidance of Dr. Prabhakar K from the department of General Medicine.

Informed Consent Form for Clinical Studies

This Informed Consent Form has two parts:

• Information Sheet (to share information about the research with you)

• Certificate of Consent (for signatures if you agree to take part)

You will be given a copy of the full Informed Consent Form

PART I: Information Sheet

Introduction

I am Dr. Likitesh A B, working under the guidance of Dr. Prabhakar K from R.L.Jalappa Research Institute. We are doing research on Type 2 Diabetes mellitus, which is most common in this country. I am going to give you information and invite you to be part of this research. You do not have to decide today whether or not you will participate in the research. Before you decide, you can talk to anyone you feel comfortable with about the research.

There may be some words that you do not understand. Please ask me to stop as we go through the information and I will take time to explain. If you have questions later, you can ask them of me, the study doctor or the staff.

Purpose of the research

Type 2 Diabetes Mellitus is one of the common medical illness and serious metabolic disorder which is rapidly raising pandemic in Indian population with early onset than our western counterparts. Diabetes causes microvascular and macrovascular complications but microvascular complications like Diabetic Retinopathy, Diabetic Nephropathy, and diabetic Neuropathy are most common.

Diabetic dyslipidemia encompasses both quantitative and qualitative changes in plasma lipids and lipoproteins, characteristically observed in individuals with type-2 diabetes mellitus.

Study on the levels of lipoprotein (a), apolipoprotein A1 and apolipoprotein B and its association with microvascular complications in type 2 DM will bring to limelight the importance and potentiate the clinical application of extended lipid profile as an early marker for microvascular complication and early treatment.

Type of Research Intervention

This research will involve collection of clinical history and necessary investigations such as clinical tests for assessment of peripheral neuropathy, funduscopic examination by ophthalmologist for assessment of retinopathy and laboratory investigations for nephropathy. Lab investigations such as complete haemogram, Fasting blood sugar, Post prandial blood sugar, HbA1C, Fasting Serum Triglycerides, Total Cholesterol, HDLc, LDLc, Fasting Lipoprotein (a) , Fasting Apolipoprotein A1, Fasting Apolipoprotein B, Blood urea, Serum Creatinine levels, Urine routine and sugar, Urine Albuminuria in a random spot collection of urine, biothesiometry.

Participant selection

We are inviting all individuals who attend outpatients and inpatients to participate in the research on study of Type 2 diabetes mellitus.

Voluntary Participation

Your participation in this research is entirely voluntary. It is your choice whether to participate or not. Whether you choose to participate or not, all the services you receive at this clinic will continue and nothing will change. If you choose not to participate in this

research project, you will offered the treatment that is routinely offered in this clinic/hospital for rheumatoid arthritis, and we will tell you more about it later. You may change your mind later and stop participating even if you agreed earlier.

Procedures and Protocol

We will be taking history, performing clinical examination and nescessarey

investigations such complete haemogram,Fasting blood sugar, Post prandial blood sugar, HbA1C, Fasting Serum Triglycerides, Total Cholesterol, HDLc, LDLc, Fasting Lipoprotein (a) , Fasting Apolipoprotein A 1, Fasting Apolipoprotein B, Blood urea, Serum Creatinine levels, Urine routine and sugar, Urine Albuminuria in a random spot collection of urine, biothesiometry will be done.

Duration

The research takes place over 1-2 days of admission or outpatient to the hospital.

Side Effects

No side effects in participating in the study.

Risks:

NO SIGNIFICANT RISK INVOLVED FOR PARTICIPATING IN THE STUDY.

Benefits

If you participate in this research, you will have the following benefits: any interim illnesses will be treated at no charge to you. There may not be any benefit for you but your participation is likely to help us find the answer to the research question. There may not be any benefit to the society at this stage of the research, but future generations are likely to benefit.

Reimbursements

You will not be given any money or gifts to take part in this research.

Confidentiality

With this research, something out of the ordinary is being done in your community. It is possible that if others in the community are aware that you are participating, they may ask you questions. We will not be sharing the identity of those participating in the research. The information that we collect from this research project will be kept confidential.

Information about you that will be collected during the research will be put away and no one but the researchers will be able to see it. Any information about you will have a number on it instead of your name. Only the researchers will know what your number is and we will lock that information up with a lock and key. It will not be shared with or given to anyone except Dr. Prabhakar K and my Co Guide Dr Shashidhar KN.

Sharing the Results

The knowledge that we get from doing this research will be shared with you through community meetings before it is made widely available to the public. Confidential information will not be shared. After these meetings, we will publish the results in order that other interested people may learn from our research.

Right to Refuse or Withdraw

You do not have to take part in this research if you do not wish to do so and refusing to participate will not affect your treatment at this clinic in any way. You will still have all the benefits that you would otherwise have at this clinic. You may stop participating in the research at any time that you wish without losing any of your rights as a patient here. Your treatment at this clinic will not be affected in any way.

Alternatives to Participating

If you do not wish to take part in the research, you will be provided with the established standard treatment available at the center/institute/hospital.

Who to Contact

If you have any questions you may ask them now or later, even after the study has started. If you wish to ask questions later, you may contact:

Dr. Likitesh A B

Postgraduate in general medicine

Sri Devaraj Urs Medical College.

Tamaka Kolar,Karnataka , Pin:563101

Ph no- 9008007471, Email.id- likitesh2010@gmail.com

This proposal has been reviewed and approved by Ethical Clearance Committee, which is a committee whose task it is to make sure that research participants are protected from harm. If you wish to find about more about the IRB. It has also been reviewed by the Ethics Review Committee of Sri Devaraj Urs Medical College, which is supporting the study.

PART II: Certificate of Consent

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a participant in this research.

Print Name of Participant_____

Signature of Participant _____

Date _____

Day/month/year

If illiterate

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of witness_____ AND Thumb print of Participant

Signature of witness _____

Date _____

Day/month/year

Statement by the researcher/person taking consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands that the following will be done:

1.

2.

3.

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this ICF has been provided to the participant.

Print Name of Researcher/person taking the consent_____

Signature of Researcher /person taking the consent_____

Date _____ (Day/month/year)

ಮಾಹಿತಿಯುಕ್ತ ಸಮ್ಮತಿಯ ನಮೂನೆ

ಟೈಪ್ 2 ಡಯಾಬಿಟಿಸ್ ಮೆಲ್ಲಿಟಸ್ ರೋಗಿಗಳು ವಿಸ್ತರಿಸಲಾಗುವುದು ಲಿಪಿಡ್ ಪ್ರೊಫೈಲ್ ಮತ್ತು

ಮೈಕ್ರೋವ್ಯಾಸ್ಕುಲರ್ ತೊಡಕುಗಳು ಪರಸ್ಪರ ಸಂಬಂಧವನ್ನು.

ಲಿಪೋಪ್ರೊಟೀನ್ಗಳು ಮಟ್ಟವನ್ನು ಅಧ್ಯಯನ, **apolipoprotien** ಎ 1 ಮತ್ತು **apolipoprotien** ಬಿ ಮತ್ತು ಟೈಪ್ 2 ಡಯಾಬಿಟಿಸ್ ಮೆಲ್ಲಿಟಸ್ ರಲ್ಲಿ ಮೈಕ್ರೋವ್ಯಾಸ್ಕುಲರ್ ತೊಡಕುಗಳನ್ನು ಸಹಯೋಗದೊಂದಿಗೆ ಪ್ರಾಮುಖ್ಯತೆಯನ್ನು ಪ್ರಚಾರದಿಂದ ಮತ್ತು ಮೈಕ್ರೋವ್ಯಾಸ್ಕುಲರ್ ತೊಡಕು ಮತ್ತು ಆರಂಭಿಕ ಚಿಕಿತ್ಸೆಗೆ ಆರಂಭಿಕ ಮಾರ್ಕರ್ ವಿಸ್ತೃತ ಲಿಪಿಡ್ ಪ್ರೊಫೈಲ್ ವೈದ್ಯಕೀಯ ಅಪ್ಲಿಕೇಶನ್ ಸಾಮರ್ಥ್ಯವನ್ನು ತರುವುದು.ನೀವು ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಒಪ್ಪುತ್ತೀರಿ ವೇಳೆ ನೀವು ಅಥವಾ ನೀವು ಅಥವಾ ಎರಡೂ ಜವಾಬ್ದಾರಿ ವ್ಯಕ್ತಿಯಿಂದ ಮಾಹಿತಿ (ಪ್ರತಿ **proforma** ಮಾಹಿತಿ) ಸಂಗ್ರಹಿಸುತ್ತದೆ . ನಿಮ್ಮ ಆಸ್ಪತ್ರೆ ದಾಖಲೆಯಿಂದ ಚಿಕಿತ್ಸೆ ಮತ್ತು ಸೂಕ್ತ ವಿವರಗಳನ್ನು ಸಂಗ್ರಹಿಸುತ್ತದೆ . ಸಂಗ್ರಹಿಸಿದ ಈ ಮಾಹಿತಿ ಮಾತ್ರ ಪ್ರೌಢಪ್ರಬಂಧದಲ್ಲಿ ಮತ್ತು ಪ್ರಕಟಣೆ ಬಳಸಲಾಗುತ್ತದೆ . ಈ ಅಧ್ಯಯನವು ಸಾಂಸ್ಥಿಕ ನೈತಿಕ ಸಮಿತಿಯು ವಿಮರ್ಶಿಸುತ್ತದೆ ಮಾಡಲಾಗಿದೆ . ನೀವು ಭಾಗವಹಿಸಲು ಇಚ್ಛಿಸದಿದ್ದರೆ ನೀವು ಪಡೆಯುತ್ತಾನೆ ಆರೈಕೆ ಬದಲಾಗುವುದಿಲ್ಲ . ನೀವು ಸ್ವಯಂಪ್ರೇರಣೆಯಿಂದ ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಒಪ್ಪಿಕೊಂಡಲ್ಲಿ ಹೆಬ್ಬೆಟ್ಟಿನ ಗುರುತು ಸೈನ್ / ಒದಗಿಸುವ ಅಗತ್ಯವಿದೆ .

ನಾನು ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಅಧ್ಯಯನದಿಂದ ಹಿಂತೆಗೆದುಕೊಳ್ಳುವಂತೆ ಮತ್ತು ಈ ನನ್ನ ಮುಂದಿನ ಆರೈಕೆ ಬದಲಾಗುವುದಿಲ್ಲ ಉಚಿತ ಉಳಿಯಲು ಎಂದು ಅರ್ಥ . ನಾನು ಓದಲು ಅಥವಾ ನನಗೆ ಓದಲು ಮಾಡಲಾಗಿದೆ ಮತ್ತು ಅಧ್ಯಯನದ ಉದ್ದೇಶ , ಬಳಸಲಾಗುವ ವಿಧಾನ , ಅಧ್ಯಯನ ಮತ್ತು ಅಧ್ಯಯನದ ಸಮಯದಲ್ಲಿ ಸಂಗ್ರಹಿಸಿದ ಮತ್ತು ಬಹಿರಂಗ ನಡೆಯಲಿದೆ ಮಾಹಿತಿಯನ್ನು ಪ್ರಕೃತಿಯಲ್ಲಿ ನನ್ನ ಒಳಗೊಳ್ಳುವಿಕೆ ಸಂಬಂಧಿಸಿದ ಅಪಾಯ ಮತ್ತು ಲಾಭಗಳನ್ನು ಅರ್ಥ . ನಾನು ಅಧ್ಯಯನ ಮತ್ತು ನನ್ನ ಪ್ರಶ್ನೆಗಳಿಗೆ ವಿವಿಧ ಅಂಶಗಳನ್ನು ನನ್ನ ತೃಪ್ತಿ ಉತ್ತರಿಸುವ ಬಗ್ಗೆ ನನ್ನ ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳಲು ಅವಕಾಶ ಹೊಂದಿದ್ದರು . ನಾನು , ಈ

ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಮತ್ತು ಪ್ರೌಢಪ್ರಬಂಧದಲ್ಲಿ ನನ್ನ ವೈಯಕ್ತಿಕ ಮಾಹಿತಿಯ ಸಂಗ್ರಹಣೆ ಮತ್ತು ಡಿಸ್ಕ್ಲೋಸರ್ ಅಧಿಕೃತಗೊಳಿಸಲು ಒಪ್ಪುತ್ತೀರಿ ರುಜುಮಾಡಿರುವ .

ವಿಷಯದ ಹೆಸರು ಸಹಿ / ಹೆಬ್ಬೆಟ್ಟಿನ ಗುರುತು

(ಪಾಲಕರು / ಗಾರ್ಡಿಯನ್ಸ್ ಹೆಸರು)

ಒಪ್ಪಿಗೆ ತೆಗೆದುಕೊಳ್ಳುವ ವ್ಯಕ್ತಿಯ ಹೆಸರು ಮತ್ತು ಸಹಿ

PATIENT INFORMATION SHEET

A COMPARATIVE STUDY OF ASSOCIATION OF EXTENDED LIPID PROFILE WITH MICROVASCULAR COMPLICATIONS IN TYPE 2 DIABETES MELLITUS.

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your GP if you wish.

Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Type 2 Diabetes Mellitus is one of the common medical illness and serious metabolic disorder which is rapidly raising pandemic in Indian population with early onset than our western counterparts. Diabetes causes microvascular and macrovascular complications but microvascular complications like Diabetic Retinopathy, Diabetic Nephropathy, and diabetic Neuropathy are most common.

Diabetic dyslipidemia encompasses both quantitative and qualitative changes in plasma lipids and lipoproteins, characteristically observed in individuals with type-2 diabetes mellitus.

Study on the levels of lipoprotein (a), apolipoprotein A1 and apolipoprotein B and its association with microvascular complications in type 2 DM will bring to limelight the importance and potentiate the clinical application of extended lipid profile as an early marker for microvascular complication and early treatment.

For the purpose of this study you will have to answer a few simple questions after which physical examination shall be carried out. For the purpose of this study necessary invasive procedures or investigations will be carried out.

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason.

This will not affect the standard of care you receive.

PROFORMA

FOR A COMPARITIVE STUDY OF ASSOCIATION OF EXTENDED LIPID PROFILE WITH MICROVASCULAR COMPLICATIONS IN TYPE 2 DIABETES MELLITUS.

Serial No -

Name: - **OP/IP:-**

Age: - **DOA:-**

S/ E Status: - **DOD:-**

Sex: - M/F **Address:-**

Occupation:-

Religion:-

1. CLINICAL HISTORY; -

A) Presenting C/O:-

1.

2.

3.

B) Details of Diabetes:

Duration-

Treatment-OHA /Insulin

Response to treatment (control)-good/fair/not controlled

C) H/O Hypertension:-

Duration-

Symptoms-

Treatment

F) Co existing disorders-CAD/Obesity

G) Breathlessness- exersional/non-exersional-grade Orthopnea +/-

PND +/- Cough: +/-

Chest pain- +/- Anginal/Non-anginal-

Parasthesia- +/- Location- Progressive +/-

Motor weakness +/- Visual disturbance +/- Stroke +/-

H) Past History –

I) F/ H (with duration)-DM/HT/IHD/Renal diseases

J) Personal History –

Smoking-Cigarette/Beedis

Alcohol – P/A Duration - Quantity -

2. CLINICAL EXAMINTION -

GPE

Build- Poor/Moderate/Heavy

Nutrition-Under/Moderate/Overweight/Obese

Pulse- B.P -

Height- Weight- BMI –

Waist cir- Hip cir- W/H Ratio-

Skin lesions +/- P.vessels D.foot +/- Arcus senilis +/- Xanthoma +/-

3. SYSTEMIC EXAMINATION -

• Abdominal examination-

• C.V.S Examination-

• Respiratory system-

• C. N. S. Examination-

H M F - Cr. Nerves-

Motor system –

Jerks – Sensory – Cerebellar –

E/O Peripheral neuropathy-

Sensory- Motor-

DTRs- Trophic ulcers-

Fundus examination –

4. INVESTIGATIONS: –

A) HAEMOGRAM

Test	Report
Hb %	
TC	
ESR	
Peripheral smear	

B) BIOCHEMISTRY

a)

Test	Report
RBS	
FBS	
PPBS	
HBAIC	
BUN	
SERUM CREATININE	

b) LIPID PROFILE

TEST	REPORT
TOTAL CHOLESTEROL	
TRIGLYCERIDES	
HDL	
VLDL	
LDL	

c) EXTENDED LIPID PROFILE

TEST	REPORT
LIPOPROTEIN (a)	
APOLIPOPROTIEN A1	
APOLIPOPROTIEN B	

d) URINE ROUTINE

TEST	REPORT
SUGAR	
PROTEIN	
MICROSCOPY	

e) SPOT URINE FOR ALBUMINURIA

Microalbuminuria – P/A

Macroalbuminuria – P/A

C) ECG –

D) USG ABDOMEN –

E) BIOTHESIOMETRY -

5. TOTAL DIAGNOSIS -

6. TREATMENT -

7. FOLLOW UP –

KEY TO MASTER CHART

1. Hosp No	Hospital Number
2. H/O HTN	History of Hypertension
3. NPDR	Non Proliferative Diabetic Retinopathy
4. PDR	Proliferative Diabetic Retinopathy
5. P	Presence of Variables
6. A	Absence of Variables
7. RBS	Random Blood Sugar
8. FBS	Fasting Blood Sugar
9. PPBS	Post Prandial Blood Sugar
10. HbA1c	Glycated Hemoglobin
11. MA	Microalbuminuria
12. HDL	High Density Lipoprotein
13. LDL	Low Density Lipoprotein
14. VLDL	Very Low Density Lipoprotein
15. Apo A1	Apolipoprotein A1
16. Apo B	Apolipoprotein B

Diabetic patients with microvascular complications

Sl No	Hosp No	AGE(YRS)	GENDER	YEARS OF DIABETES	TREATMENT	H/O HTN	FAMILY H/O DIABETES	H/O SMOKING	H/O ALCOHOL	BMI(Kg/m^2)	RETINOPATHY	TYPE	NEPHROPATHY	NEUROPATHY	SEVIARITY	RBS	FBS	PPBS	HBA1C	MA(Mg/L)	CHOLESTEROL	TRIGLYCERIDE	HDL	LDL	VLDL	LIPOPROTIEN (a)	APO A1	APO B
1	172170	86	M	20	INSULIN	P	A	P	P	26.6	P	NPDR	P	P	2	143	126	185	8.5	190	177	94	24	213	58.3	56	129	89
2	179993	85	M	8	INSULIN	P	P	A	A	27.9	P	NPDR	P	A		200	198	289	9.5	59	175	165	33	105	42.1	68.6	189	69
3	176680	66	F	10	OHA/INSULIN	A	A			28.9	A		P	P	3	123	167	182	7.8	35	249	169	37	178	23.2	27	109	145
4	179980	52	F	20	OHA/INSULIN	P	A			25.9	P	NPDR	P	A		245	229	281	11.4	46	210	140	45	237	34.4	35	176	123
5	175143	70	F	10	INSULIN	A	A			27.8	P	NPDR	P	P	1	112	154	220	10.2	59	155	145	30	76.5	42.2	78.8	198	73
6	7319	72	M	6	INSULIN	P	A	P	P	21.9	P	NPDR	P	A		200	138	211	11	165	190	228	39	105	51.9	58	54	78
7	180014	55	M	10	OHA	P	P	P	A	29.9	P	NPDR	P	P	1	120	101	156	6.6	30	124	186	41	102	43.4	98	176	89
8	241387	66	M	7	OHA	P	A	A	A	19.6	A		P	A		98	110	116	8.9	30	133	211	43	90	24.1	34	90	76
9	240883	60	F	5	OHA	P	P			22.5	A		P	A		154	123	205	7.8	60	154	156	36	97	24.8	56	154	45
10	230986	65	M	9	OHA	A	A	P	A	21.6	P	PDR	P	A		234	246	354	12.5	60	198	167	45	81	63.7	5.43	198	98
11	169397	60	M	18	OHA/INSULIN	A	A	A	P	24.5	A		P	P	2	87	157	104	10.4	75	156	155	40	149	56.1	32	154	134
12	172197	80	M	6	OHA	P	P	A	P	26.6	P	NPDR	P	A		123	171	189	8.5	37	132	178	50	122	54.7	34	123	111
13	164779	50	F	9	OHA/INSULIN	A	A			32.1	A		P	A		245	164	377	9.1	71	154	189	46	123	42.1	45	73	90
14	148655	65	F	10	INSULIN	P	P			28.9	P	PDR	P	P	3	289	262	307	10.7	190	176	100	40	192	36.4	12.4	178	80
15	242153	65	F	6	OHA	P	A			26.7	A		P	P	2	167	110	250	7	34	124	186	34	103	38.3	65.4	165	89
16	238499	60	F	10	OHA/INSULIN	A	A			25.3	A		P	A		234	196	302	11.2	82	143	122	28	198	39.6	23	175	76
17	232994	50	M	5	OHA	P	A	P	A	24.5	P	PDR	P	P	1	154	129	320	8.5	43	176	175	40	96	56.3	18.3	76	98
18	215166	60	M	8	OHA	P	A	P	A	22.4	A		P	A		78	95	117	6.2	190	165	146	33	123	52.5	36	165	99
19	222111	50	M	7	OHA	A	P	P	P	26.8	A		P	A		89	147	289	9.4	190	142	126	40	78.4	34.6	45.7	98	111
20	130837	68	M	8	OHA/INSULIN	A	A	A	A	28.9	P	NPDR	P	A		189	200	346	7	41	350	149	25	198	34.8	26.5	89	156
21	223273	75	M	10	INSULIN	A	A	A	A	23.8	P	NPDR	P	A		267	134	354	8	43	146	148	36	119	58.4	32	111	143
22	225115	60	M	10	OHA/INSULIN	A	A	P	P	34.8	P	NPDR	P	P	1	165	192	320	9.2	61	145	177	34	104	34.8	24	125	87
23	161271	57	M	10	INSULIN	A	A	A	P	22.7	A		P	P	1	112	156	289	7.5	146	187	185	26	146	62.8	56	115	76
24	227502	63	M	9	OHA	P	A	A	P	23.8	A		P	A		298	307	491	7.8	51	188	173	30	140	66.8	73	134	65
25	227911	70	F	15	OHA/INSULIN	P	A			26.6	A		P	P	2	105	135	298	12.9	60	164	124	42	178	52.5	34	98	89
26	227919	55	M	5	OHA	P	A	P	P	26.4	P	NPDR	A	A		165	112	345	7.5	12	133	142	22	82.7	28.3	7.7	102	85
27	132110	52	M	5	OHA	P	A	A	A	29.9	P	PDR	P	P	3	115	132	234	6.9	45	134	187	16	75.9	42.1	17.3	66	83
28	228835	60	M	12	INSULIN	A	A	P	A	27.8	A		P	A		154	145	256	8.3	60	105	140	20	61.8	23.2	23	82	72
29	229170	48	F	8	OHA	A	P			24.8	P	PDR	P	A		143	165	254	7.4	156	89	232	17	37.6	34.4	6	77	76
30	217530	45	M	7	INSULIN	A	A	A	A	25.8	P	NPDR	A	P	3	178	172	358	11	6	166	180	30	114	22.2	12.5	112	91
31	229877	42	M	5	OHA	A	P	P	A	22.9	P	NPDR	A	A		187	182	289	9.4	10	116	177	18	66.1	31.9	19.6	168	146
32	232140	38	M	8	INSULIN	A	P	P	A	27.8	A		P	A		234	165	346	7	98	105	176	11	151	43.4	8.2	130	92
33	1021069	62	F	7	OHA	P	A			28.8	P	NPDR	P	P	2	154	154	234	8	120	127	112	40	62.9	24.1	12.5	119	77
34	136028	85	M	8	OHA	P	A	P	P	23.8	P	NPDR	P	P		156	145	345	11	76	97	295	9	157	24.8	3.3	30	78
35	232415	79	F	20	OHA	P	A			22.7	A		P	A		178	187	236	7.8	84	165	166	47	94.3	23.7	81.6	140	86
36	197699	72	M	7	INSULIN	A	A	A	A	21.8	P	NPDR	A	P	1	165	134	345	9.1	8	147	138	46	84.9	16.1	25	135	78
37	234607	70	F	11	OHA	A	A			23.3	P	NPDR	P	P	2	189	123	267	9.4	123	180	90	34	131	34.7	28.6	102	96
38	234714	70	M	12	OHA	A	A	P	A	23.7	P	NPDR	P	A		178	154	239	7.6	230	132	80	24	95.9	12.1	78.4	188	122
39	227030	65	M	8	OHA	P	A	P	A	21.8	P	NPDR	A	A		123	165	226	6.8	12	156	131	41	109	6.4	19.3	127	85
40	235506	65	F	9	INSULIN/OHA	P	A			29.9	A		A	P	1	178	110	228	7.3	6	161	205	34	88.7	38.3	22.7	118	89
41	227925	60	M	8	INSULIN	P	A	P	A	23.5	P	PDR	P	P	3	156	117	265	6.8	167	131	251	24	67.4	39.6	79	102	85
42	77007	60	F	6	INSULIN	A	A			22.4	A		A	P	2	200	98	254	7.2	5	132	194	24	81.7	26.3	3	190	82
43	278362	75	M	12	INSULIN	A	A	A	A	24.4	P	NPDR	P	P	2	234	124	253	7.8	34	87	210	19	36.9	12.5	12	145	96
44	337332	75	M	7	INSULIN	P	A	A	A	23.7	A		P	A		289	176	256	6.3	98	161	73	63	83.3	34.6	16	66	83
45	278248	50	F	8	OHA	A	P			26.5	P	PDR	P	P	2	234	154	321	7.9	68	161	73	43	83.5	34.8	6.9	82	72
46	68112	70	M	7	OHA/INSULIN	P	A	P	P	23.6	P	NPDR	A	A		165	143	231	6.9	12	228	210	20	36.7	28.4	23	77	76
47	189883	55	M	5	OHA/INSULIN	A	A	P	P	23.5	A		P	A		117	136	386	10.1	176	368	210	26	56.9	34.8	34	112	91
48	29096	58	M	6	OHA	A	A	A	P	30.3	P	NPDR	A	P	2	173	123	243	7.8	12	287	210	20	86.9	32.8	56	68	76
49	158180	68	M	6	OHA	P	A	A	A	24.3	P	NPDR	A	P	2	154	145	289	14	15	124	106	44	58.7	6.8	23	34	92
50	337099	76	M	8	OHA/INSULIN	P	A	P	P	22.8	P	NPDR	P	A		245	165	298	6.7	34	280	210	24	121	12.5	32	119	140
51	159166	72	M	11	INSULIN	P	A	A	P	32.8	P	NPDR	P	A		234	92	143	8.7	73	161	73	63	83.6	34.9	45.8	180	78
52	194785	65	F	6	OHA/INSULIN	A	A			34.7	A		A	P	2	245	145	268	9.5	12	200	180	40	110	18.8	16.8	123	89
53	41675	62	M	8	OHA/INSULIN	A	A	A	A	26.6	P	NPDR	P	A		230	171	256	8.8	67	151	70	63	83.6	15.9	15	135	78
54	290535	67	M	8	OHA	P	A	P	A	21.5	A		P	A		126	162	311	10.2	56	164	70	63	83.9	18.9	14.7	156	98
55	329090	60	M	9	OHA/INSULIN	P	A	A	A	24.7	P	NPDR	P	P	2	145	187	354	7.8	109	160	70	30	181	458	54	88	76
56	327532	70	M	8	OHA	A	A	P	P	23.2	P	NPDR	P	A		156	109	234	9.6	60	173	79	53	104	34.7	32.9	132	87
57	327392	60	M	10	OHA	A	A	P	A	25.7	A		A	P	2	189	110	265	9.3	23	186	86	48	114	23.8	24	118	89
58	339138	45	F	8	OHA	P	P			30.9	P	NPDR	P	P	3	198	114	245	8.3	45	164	82	34	174	47.9	33.7	179	89
59	218297	56	F	12	OHA/INSULIN	P	A			25.9	P	NPDR	P	P	2	157	156	321	11	158	182	178	30	96.5	56.2	24.9	78	126
60	279699	75	M	13	OH/INSULIN	P	A	A	A	22.3	A		A	P	1	146	165	298	7.8	23	200	268	36	80.4	44.8	13.8	176	56
61	171964	64	M	9	OHA	A	A	P	P	24.7	P	PDR	P	A		234	187	276	10.4	130	205	277	44	106	58.2	16.9	123	76
62	171982	80	M	20	OHA	A	A	P	A	24.8	P	NPDR	P	P	3	221	123	256	9.9	98	196	158	35	129	64.8	19.5	178	87
63	286716	70	M	14	OHA/INSULIN	A	A	A	P	27.3	P	NPDR	P	P	3	145	176	345	10.6	190	239	233	42	152	34.7	35	123	74
64	324534	62	M	6	OHA	P	A	A	A	25.9	P	NPDR	A	P	1	142	154	234	7.5	15	190	228	39	104	44.9	12.9	69	59
65	395673	55	M	6	OHA	A	P	P	A	24.8	P	NPDR	A	A		175	98	234	6.6	21	251	198	40	173	52.4	14.9	121	82

Patients without microvascular complications

Sl No	Hosp No	AGE(YRS)	GENDER	YEARS OF DIABETES	TREATMENT	H/O HTN	F/ H DIABETES	H/O SMOKING	H/O ALCOHOL	BMI(Kg/m 2)	RETINOPATHY	NEPHROPATHY	NEUROPATHY	RBS	FBS	PPBS	HBA1C	MA(Mg/L)	CHOLESTEROL	TRIGLYCERIDE	HDL	LDL	VLDL	LIPOPROTIEN (a)	APO A1	APO B
1	180204	67	M	5	OHA	P	A	P	A	23.6	A	A	A	234	132	220	5.6	6	177	94	45	113	28.3	7.7	102	87
2	240675	57	M	6	OHA	A	A	A	A	17.9	A	A	A	342	132	234	6.9	7	175	165	43	105	42.1	17.3	66	76
3	179523	75	M	7	OHA	P	A	A	P	28.9	A	A	A	221	143	256	6.6	13	129	169	37	178	23.2	23	82	72
4	180401	48	F	5	OHA	A	A			25.9	A	A	A	210	165	254	7.4	12	210	140	45	137	34.4	6	77	78
5	177916	65	M	8	OHA	A	P	A	P	20.8	A	A	A	345	123	358	5.6	24	155	145	50	76.5	22.2	12.5	100	91
6	178182	60	M	9	INSULIN	A	A	A	A	28.9	A	A	A	124	182	289	9.4	16	130	228	39	105	31.9	19.6	68	56
7	170921	60	M	5	OHA	A	A	P	A	19.9	A	A	A	234	165	346	7	7	124	186	41	102	43.4	8.2	30	65
8	171306	65	F	7	OHA	A	A			23.6	A	A	A	145	123	234	5.6	8	133	211	43	90	24.1	12.5	119	77
9	167911	60	M	7	OHA	A	A	P	P	22.5	A	A	A	92	145	345	6	23	154	156	36	97	24.8	3.3	30	78
10	240646	70	F	9	OHA	P	A			21.6	A	A	A	145	156	236	7.8	16	198	167	45	81	23.7	81.6	140	86
11	153328	53	M	5	OHA	A	A	P	A	24.5	A	A	A	134	134	345	9.1	6	156	155	40	149	16.1	25	110	78
12	172529	80	F	6	OHA	P	A			23.6	A	A	A	89	156	267	4.8	4	132	178	50	122	14.7	28.6	102	67
13	180250	51	M	7	OHA	A	A	A	P	32.1	A	A	A	105	154	239	7.6	5	154	189	46	123	12.1	78.4	88	72
14	175143	45	M	5	OHA	A	A	A	P	19.9	A	A	A	123	134	226	6.8	12	176	100	40	92	6.4	19.3	102	85
15	243096	35	F	9	INSULIN	P	P			17.7	A	A	A	165	123	228	7.3	11	124	86	34	102.5	38.3	22.7	118	89
16	178371	43	M	8	OHA	A	A	P	A	23.3	A	A	A	145	120	265	6.8	17	143	122	58	98	39.6	79	102	85
17	178625	40	M	6	OHA	A	A	A	A	24.5	A	A	A	167	98	254	7.2	9	176	75	40	96	26.3	3	90	82
18	215166	75	M	5	OHA	P	A	P	A	22.4	A	A	A	178	145	253	7.4	7	165	146	33	93	12.5	12	120	96
19	222111	75	M	8	INSULIN	P	A	A	A	26.8	A	A	A	189	176	256	6.3	6	142	126	40	78.4	34.6	16	66	55
20	130837	50	F	5	OHA	A	A			21.9	A	A	A	142	165	321	6.4	18	150	149	45	198	14.8	6.9	82	72
21	223273	70	M	5	OHA	P	A	A	A	19.8	A	A	A	98	143	231	6.9	7	146	148	36	119	28.4	23	77	76
22	225115	55	M	7	OHA	A	P	A	P	22.8	A	A	A	145	143	276	6.5	6	145	77	34	104	34.8	34	112	76
23	161271	58	M	9	OHA	P	A	P	A	22.7	A	A	A	134	123	243	6.3	6	187	185	36	146	12.8	56	68	76
24	227502	68	M	6	OHA	A	A	A	A	23.8	A	A	A	154	145	289	7	23	188	173	50	140.4	6.8	23	34	92
25	227911	76	M	8	INSULIN	P	P	P	A	22.6	A	A	A	89	165	270	6.7	25	164	124	42	78	12.5	32	119	75
26	227919	72	M	7	OHA	P	A	A	P	26.4	A	A	A	98	92	143	7.9	24	196	128	39	106	34.9	45.8	30	56
27	132110	65	F	5	OHA	A	A			29.9	A	A	A	94	145	268	4.5	12	152	159	31	181	18.8	16.8	123	89
28	228835	62	M	5	OHA	A	A	P	A	27.8	A	A	A	234	145	256	6.1	7	124	187	31	92	15.9	15	102	75
29	229170	67	M	8	OHA	A	P	P	A	24.8	A	A	A	126	162	311	7.6	8	151	98	30	170	18.9	14.7	89	98
30	217530	60	M	9	OHA	A	A	A	P	25.8	A	A	A	120	187	189	7.8	6	189	137	41	79	18	54	88	76
31	229877	76	M	8	OHA	P	A	A	A	22.9	A	A	A	65	109	234	6.8	13	148	166	34	80	34.7	32.9	121	76
32	232140	67	M	7	OHA	A	A	P	P	27.8	A	A	A	98	110	265	6.5	24	248	98	32	176	23.8	24	118	89
33	1021069	43	F	8	OHA	A	P			18.8	A	A	A	84	114	245	8.3	12	105	82	34	73.6	17.9	33.7	109	89
34	136028	45	F	6	OHA	A	A			23.8	A	A	A	56	156	321	7.9	23	127	178	45	96.5	16.2	24.9	78	76
35	232415	65	M	5	OHA	A	A	A	A	22.7	A	A	A	154	165	298	7.8	12	97	68	56	80.4	24.8	13.8	165	56
36	197699	68	M	9	INSULIN/OHA	P	A	P	P	21.8	A	A	A	104	156	276	6.9	23	165	277	35	106.3	18.2	16.9	123	76
37	234607	76	M	7	OHA	P	A	A	A	23.3	A	A	A	121	123	256	6.9	23	147	158	22	129.2	34.8	19.5	154	34
38	234714	54	M	5	OHA	A	P	A	A	23.7	A	A	A	130	176	345	7.8	12	180	133	34	151.5	34.7	35	123	74
39	227030	65	M	7	OHA	A	A	P	P	21.8	A	A	A	134	154	234	7.5	14	132	228	23	104.3	14.9	12.9	69	59
40	235506	53	M	8	INSULIN	A	A	P	A	19.9	A	A	A	153	89	234	6.6	17	156	89	32	172.6	12.7	14.9	121	82
41	134675	58	M	5	OHA	p	A	P	P	23.5	A	A	A	80	119	126	5.6	11	133	142	22	82.7	28.3	12.9	87	145
42	256473	60	M	8	OHA	A	A	A	A	22.4	A	A	A	82	287	329	11.7	10	134	187	16	75.9	42.1	29.9	65	176
43	376542	45	F	4	OHA	A	A			21.4	A	A	A	78	55	125	8	12	105	140	40	61.8	23.2	34.6	70	73
44	289763	62	F	1	OHA	P	A			23.7	A	A	A	102	130	160	10.5	10	89	132	17	37.6	34.4	11.8	76	78
45	276532	65	F	3	OHA	A	A			26.5	A	A	A	80	182	320	8	10	166	80	60	113.8	22.2	23.7	89	89
46	289826	47	M	9	INSULIN	A	A	P	A	23.6	A	A	A	78	158	268	9	11	116	177	48	66.1	31.9	23.9	98	76
47	274467	60	M	15	INSULIN/OHA	A	P	P	A	23.5	A	A	A	80	214	189	10.3	11	105	76	41	50.6	43.4	34.9	54	45
48	379879	72	M	1	OHA	A	A	A	P	20.3	A	A	A	113	202	203	5.5	14	127	112	40	62.9	24.1	25	68	98
49	13880	65	F	15	INSULIN/OHA	P	A			24.3	A	A	A	78	127	58	7.8	13	97	95	49	56.6	24.8	36	78	134
50	267644	55	M	20	INSULIN/OHA	A	A	A	P	22.8	A	A	A	110	172	153	9.8	23	75	66	47	94.3	23.7	45	100	111
51	398744	43	F	15	INSULIN	A	A			22.8	A	A	A	100	53	102	12.2	20	147	98	46	84.9	16.1	24.9	87	90
52	136448	45	F	15	INSULIN	P	P			18.7	A	A	A	86	92	77	12.6	22	180	90	34	131.3	14.7	12.8	76	80
53	1567	40	M	18	INSULIN	A	A	P	P	26.6	A	A	A	82	144	256	13.2	18	132	80	24	95.9	12.1	11.9	123	89
54	39744	45	M	6	OHA	A	A	A	P	21.5	A	A	A	88	220	431	7.2	23	156	131	41	108.6	6.4	16	145	76
55	28763	56	F	15	INSULIN/OHA	A	A			24.7	A	A	A	90	147	263	14	26	61	105	34	88.7	38.3	12.8	76	98
56	38658	42	M	6	INSULIN	A	P	A	A	23.2	A	A	A	90	139	233	7.5	28	131	151	34	67.4	39.6	17.8	65	99
57	399901	45	F	1	OHA	P	A			25.7	A	A	A	120	292	264	7.8	28	102	94	54	81.7	26.3	23.7	98	111
58	397761	65	F	2	OHA	A	A			23.9	A	A	A	85	156	144	7.6	22	87	123	19	36.9	12.5	34.8	86	156
59	93764	30	M	1	OHA	A	A	P	P	21.9	A	A	A	80	156	368	8.9	6	61	73	63	83.3	34.6	35.7	80	143
60	127372	35	M	5	OHA	P	P	P	P	22.3	A	A	A	80	153	225	9.7	14	161	73	63	83.5	14.8	23.9	65	87
61	398765	50	M	1	OHA	P	A	A	A	24.7	A	A	A	120	240	270	11.4	6	128	140	20	36.7	28.4	12	76	76
62	165474	50	M	1	OHA	P	P	A	P	22.8	A	A	A	80	147	250	8.3	6	168	145	26	56.9	34.8	22.6	89	65
63	18688	50	M	7	INSULIN	A	A	P	A	27.3	A	A	A	80	124	320	10.4	6	187	120	20	86.9	12.8	21.7	87	89
64	367574	60	M	12	INSULIN	P	A	A	P	22.1	A	A	A	78	166	333	8.5	12	124	106	44	58.7	6.8	7.6	72	85
65	248478	60	M	10	INSULIN	A	A	P	P	24.8	A	A	A	90	147	275	8.5	6	180	110	44	120.8	12.5	18.9	78	83