DISSERTATION ON

STUDY OF LIPID PEROXIDATION IN DIABETES MELLITUS

SUBMITTED FOR

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CERTIFICATE

This is to certify that dissertation entitled '**STUDY OF LIPID PEROXIDATION IN DIABETES MELLITUS'** the bonafide record of workdone by **Dr.S.SUMATHI** in the Department of Biochemistry, Thanjavur Medical College, Thanjavur during her post graduate course from 2008 to 2011. This is submitted as partial fulfillment for the requirement of M.D. Degree examinations to be held in April 2011.

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LIST OF ABBREVIATIONS

DM	Diabetes Mellitus
IDDM	Insulin Dependent Diabetes
NIDDM	Non-Insulin Dependent Diabetes
MDA	Malondialdehyde
HbA1C	Hemoglobin A1C
MODY	Maturity Onset Diabetes of Young
DNA	Deoxyribonucleic Acid
GDM	Gestational Diabetes Mellitus
Glu T	Glucose Transporter
NEFA	Non Esterified Fatty Acid
TAG	Triacyl Glycerol
FA	Fatty Acid
LDL	Low Density Lipoprotein
HDL	High Density Lipoprotein
FFA	Free Fatty Acid
VLDL	Very Low Density Lipoprotein
ROS	Reactive Oxygen Species
AGE	Advanced Glycation Endproduct
TBARS	Thio Barbituric Acid Reactive Substances
TBA	Thio Barbituric Acid
HbAo	Hemoglobin Ao

CONTENTS

INTRODUCTION

Diabetes mellitus is a group of metabolic disease characterized by hyperglycaemia resulting from defects in Insulin secretion, Insulin action or both[1]. It is a complex disease where carbohydrate, protein and fat metabolism is impaired[2].

Diabetes is an "ice berg" disease. The number of cases of diabetes world wide is estimated to be around 150 million. It is estimated that 20 percent of the current global diabetic population resides in South- East Asia Region.

In India, the prevalence of disease in adults was found to be 2-4 percent in rural and 4 – 11.6 percent in urban dwellers. High frequencies of impaired glucose tolerance, shown by studies ranging from 3.6-9.1 percent indicate the potential for further rise in prevalence of DM in the coming decades[3].

CLASSIFICATION[4]

Type 1 Diabetes

A. Immune mediated

B. Idiopathic

Type 2 Diabetes

Other specific types

Gestational Diabetes Mellitus (GDM)

Impaired Glucose Tolerance (IGT)

IDDM onset is typically abrupt[5] and is usually seen in individual less than 30 years. Immune mediated and β cells of pancreas are destroyed, usually associated with ketosis, Exogenous insulin is required to reverse the catabolic state. NIDDM is more common than IDDM, gradual in onset and occurs mainly in the middle aged and elderly[3].

Diabetes is better known for its complications affecting the vascular system, kidney, retina, lens, peripheral nerves and skin which are extremely costly in terms of longevity and quality of life[6].

Lipid peroxidation is elevated in Diabetes[7]. Diabetes is usually accompanied by increased production of free radicals or reactive oxygen species[7] which produces oxidative stress. The occurrence of free radical induced lipid peroxidation causes considerable change in the cell membrane[8]. Peroxidation of Lipid membrane has been related to the pathogenesis of many degenerative diseases such as Atherosclerosis[9]. Atherosclerosis is the most common complication of diabetes[10].

Free radicals damage lipids by initiating a process called Lipid peroxidation[11].

The decomposition of lipid peroxides forms many cytotoxic compounds like malondialdehyde(MDA).

So oxidative stress can be measured by monitoring the changes in malondialdehyde[6,7]. Degree of lipid peroxidation was measured in terms of MDA.

AIM OF THE STUDY

1. To study the level of lipid peroxide in IDDM and NIDDM.

2. To find out the correlation between Lipid peroxide and Lipid profile in both types of diabetes mellitus.

3. To find out the correlation between lipid peroxide with glycaemic control (HbA1C)

REVIEW OF LITERATURE

Diabetes was described more than 2000 years ago. Aretaeus of Cappadocia (about 150 AD) described the disease and referring to the polyuria, gave the name 'DIABETES' which comes from the Greek word meaning "To run through" (Dia-Through, Bainein-To go) because he observed that the disease consisted of "a liquefaction of flesh and bone in to urine".

In 1674, Thomas Willis discovered (by tasting) that the urine of diabetic person was sweet, "As if imbued with honey (MELLITUS)."

Long before the discovery of insulin, physicians noticed that patients with diabetes fall into two clinical categories, young patients with an intolerable thirst and rapid weight loss. In contrast, older patients often over weight, presented with milder symptoms and could survive for many years with careful diet. In the 1930's Himsworth[12] observed that young thin patients were sensitive to the action of injected insulin, where as older and fatter patients were not. From this he currently inferred that one type of diabetes was due to insulin deficiency and other to insulin

insensitivity. The term type 1 and type 2 were introduced by Lister[13] in 1951.

Diabetes mellitus is a clinical syndrome characterized by hyperglycaemia due to absolute or relative deficiency of Insulin. Lack of insulin affects metabolism of carbohydrate, protein and fat[14].

AETIOLOGICAL CLASSIFICATION OF DM[15]

I. Type 1 DM
   a. Immune mediated – Type1A
   b. Idiopathic – Type1B.

II. Type 2 DM

III. Other specific types
   A. Genetic defects in β cell function
      1. MODY 1,2,3,4,5,6.
      2. Mitochondrial DNA mutation.
      3. Proinsulin to insulin conversion defect
   B. Genetic defects in Insulin action
      1. Type of insulin resistance
      2. Lipodystrophy

C. Diseases of the exocrine pancreas

Pancreatitis, Pancreatectomy.

Neoplasia, cystic fibrosis

Haemochromatosis, Fibrocalcific Pancreatopathy.

D. Endocrinopathies

Acromegaly, Cushing's Syndrome

Glucagonoma, Pheochromocytoma

Hyperthyroidism

E. Drugs or chemical induced

β adrenergic agonist

β blocker, Glucocorticoids

Pentamidine, Phenytoin,

Protease inhibitor, Thyroid hormone

Thiazides.

F. Infections

Congenital Rubella, Cytomegalovirus

G. Uncommon forms of Immune mediated diabetes

Anti-Insulin receptor Ab

H. Other genetic syndrome associated with DM

Downs, Klinefelter's, Turner's ,

Wolf syndrome, Friedreich's ataxia, Huntington's chorea, Porphyria.

IV. GDM

Type I DM

IDDM (Insulin Dependent Diabetes Mellitus)

Caused by deficiency of pancreatic β cells. Usually results from an auto immune response that selectively destroys pancreatic β cells[16]. Frederick Banting & Charles Best first demonstrated in 1921; that daily insulin injection to is required to survive in IDDM[17]. There are two types, one is immune mediated & other is idiopathic[18].

NIDDM (Non Insulin Dependent Diabetes Mellitus)

Unlike IDDM this is relatively common in all populations enjoying an affluent life style. The disease may be present in a subclinical form for years before diagnosis and the incidence increase markedly with age and degree of obesity. The onset may be accelerated by stress of pregnancy, drug treatment or intercurrent illness[19]. Insulin resistance is considered as an important pathophysiological defect in the development of type 2 diabetes[20,21] along with β cell function[22].

Blood glucose concentrations are maintained within normal limits in healthy people by insulin. This insulin is secreted from β cells of pancreas.

Circulating glucose derived from three main sources.

1. The gut, as a result of hydrolysis or hepatic conversion of a variety of ingested carbohydrates.
2. Hepatic and some other glycogen stores (Glycogenolysis).
3. New synthesis from precursors (Gluconeogenesis).

Insulin Secretion and action[11]

Glucose concentration is the key regulator of insulin action.

The principal antihyperglycaemic actions of insulin are

a) Insulin reduces the production of gluconeogenic precursors such as glycerol, alanine and lactate
b) reduces activity of hepatic gluconeogenic enzyme.
c) increases hepatic glycogenolysis to glucose.
d) reduced hepatic glucose output.
e) increase cellular glucose uptake mediated by GLUT4.
f) reduces competition for glucose oxidation by alternative fuels.
g) initiation of NEFA release from adipose tissue.

h) reduces hepatic ketogenesis.

i) insulin promotes glucose storage as glycogen.

In diabetes due to deficiency of insulin, despite high blood glucose levels, cells 'starve' since insulin stimulated glucose entry into cells is impaired. TAG hydrolysis, FA oxidation, gluconeogenesis and ketone body formation are accelerated[17].

METABOLIC DEFECTS IN DIABETES

Lack of insulin leads to mobilization of substances for gluconeogenesis and ketogenesis from muscle and adipose tissue, accelerated production of glucose and ketone by the liver and impaired removal of endogenous and exogenous fuels by insulin responsive tissues. The net results are severe hyperglycaemia and hyperketonemia that overwhelm renal removal mechanism[23].

Insulin affects many sites of mammalian lipids metabolism. It stimulates synthesis of FA in liver, adipose tissue and in the intestine. Insulin increases cholesterol synthesis and the activity of lipoprotein lipase activity in white adipose tissue is increased[24].

DYSLIPIDAEMIA IN DM

Common form of dyslipidaemia in DM is that hypertriglyceridaemia with reduced HDL levels [25]

Once diabetes has developed, increased concentrations of LDL cholesterol and decreased concentrations of HDL cholesterol appear. Elevated serum triglycerides with low HDL cholesterol and increased LDL are common in type 2 diabetic patients without significant hypercholesterolemia[26].

In diabetes due to absence of insulin, hormone sensitive lipase is activated, more FFA are formed, these are catabolised to produce acetyl COA. As available oxaloacetate is less, acetyl COA is not readily utilized. So increased Acetyl COA is channelled to cholesterol synthesis leading to increased serum cholesterol levels[27].

Hormone sensitive lipase hydrolyses triglycerides to glycerol and fatty acids[28]. The activity of endothelial insulin dependent lipoprotein lipase activity is less resulting in diminished triglyceride clearance from triglyceride rich lipoproteins. This results in hypertriglyceridemia.

The low lipoprotein lipase activity results in impaired lipolysis of VLDL and reduced formation of HDL particles[29].

OXIDATIVE STRESS IN DM

Lipid peroxidation is elevated in diabetes[30]. Diabetes mellitus is considered to be rank one of free radical disease which propagates complications with increased free radical formation. Lipid peroxides are non-radical intermediates derived from unsaturated fatty acids, phospholipids, glycolipids, cholesterol esters and cholesterol. This formation occurs in enzymatic or non-enzymatic reactions involving activated chemical species known as “reactive oxygen species” (ROS) which are responsible for toxic effects in the body via various tissue damages.

Excessively high levels of free radicals cause damage to cellular proteins, membrane lipids and nucleic acids and eventually cell death.

Glucose oxidation is the main source of free radicals. Glucose in its enediol form is oxidized in a transition-metal dependent reaction to an enediol radical anion that is converted into reactive ketoaldehydes and to

superoxide anion radicals. The superoxide anion radicals undergo dismutation to hydrogen peroxide.

H_2O_2 if not degraded by catalase or glutathione peroxidase and in the presence of transition metals, can lead to production of extremely reactive hydroxyl radicals.

Hyperglycaemia also promotes lipid peroxidation of LDL by superoxide dependent pathway resulting in the generation of free radicals.

Damage to protein is important because it affects the function of receptors, enzymes, transport proteins and by generating new antigen that provokes immune responses[29].

Glucose interacts with protein leading to the formation of an amadori product and advanced glycation end products(AGE). AGEs via their receptors inactivate enzymes, alter their structure and function and promote free radical formation.

Prolonged oxidative stress can lead to depletion of essential antioxidants[31,32].

Imbalance between protective antioxidants and increased free radical production leading to oxidative damage is known as oxidative stress[33].

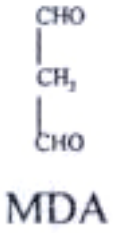
Lipid peroxidation is the free radical damage of lipids.

During lipid peroxidation of polyunsaturated fatty acids MDA is formed, by the action of human platelet thromboxane synthetase on prostaglandins PGH_2, PGH_3 and PGG_2, and by the action of polyamine oxidase and amine oxidase on spermine.

MDA is a dialdehyde and is a very reactive molecule. Under physiological conditions MDA exists as an enolate anion(O—CH=CH—CHO), a form that is only fairly reactive, forming Schiff base with molecules containing a free amine group. Under more acidic conditions(PH<4), beta hydroxyacrolein (HO—CH=CH—CHO) is the predominant form. Beta hydroxyacrolein is a very reactive electrophile capable of reacting in a Michael addition with a number of biologically important nucleophiles. Proteins are more reactive with MDA than free aminoacids forming a variety of adducts and cross-links. MDA can also react with DNA bases producing a variety of mutagenic compounds. MDA has the potential to induce amino-imino-propen cross-links between

complimentary strands of DNA and can also cause the formation of DNA- protein cross-links.

MDA is metabolized in the liver to malonic acid semialdehyde. This is unstable and spontaneously decomposes to acetaldehyde that is then converted to acetate by aldehyde dehydrogenase and inally to carbon dioxide and water. Some MDA eventually ends up as acetyl-CoA. Mammalian urine also contains enaminals derived from the hydrolysis of MDA modified proteins.



MDA

(1,1′,3,3′ Tetramethoxy propane)

MDA is used as an index of oxidative stress and is a marker of lipid oxidation[33-36]. Lipid peroxidation is important because it contributes to the development of atherosclerosis[37-39].

The study is undertaken to evaluate the relationship of lipid peroxide with lipids, lipoprotein fractions in IDDM and NIDDM to find the possibilities of preventing complications.

MATERIAL AND METHODS

Participants of the study group were selected from the outpatients population of Department of Diabetology, Thanjavur Medical College, Thanjavur.

100 patients were selected for this study. Out of which 50 patients belong to NIDDM and 50 to IDDM group.

50 persons served as healthy control.

INCLUSION CRITERIA

All ambulatory NIDDM and IDDM patients without any complications.

EXCLUSION CRITERIA :

Smokers

Alcoholics

Renal failure

Bronchial Asthma

History Suggestive of Complications of DM

- Angiopathy
- Cardiopathy

- Retinopathy
- Nephropathy

Detailed history and complete clinical examination was done in all the cases.

For all the patients, fasting and post prandial blood samples and fasting urine samples were collected. For blood sugar estimation, blood collected in fluorinated tube. For other investigations in plain tube samples were collected.

The following investigations were done.

1. Serum malondialdehyde.
2. Blood sugar
    a. Fasting
    b. Post prandial
3. HbA1C
4. Serum Lipid profile
5. Blood urea
6. Serum Creatinine
7. Urine Albumin & Sugar

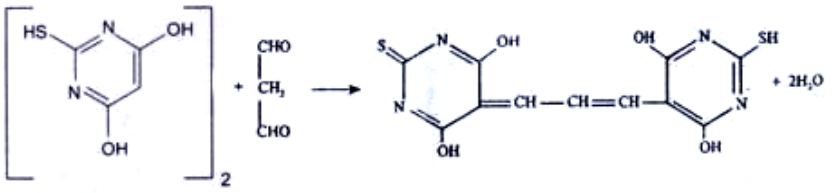
ESTIMATION OF PLASMA TBARS

METHODOLOGY:

METHOD OF YAGI

PRINCIPLE:

Reaction of MDA with Thiobarbituric acid (TBA) yields a red MDA-TBA adduct. The product of 2 mol of TBA plus 1 mol of MDA. The coloured complex is readily extractable into organic solvents such as Butanol. Quantification is done spectrophotometrically at 532 nm.



REAGENTS:

1. Sulphuric acid 0.083 N
2. Phosphotungstic acid 10%
3. n Butanol
4. Thiobarbituric acid 670 mg is dissolved in 100ml of water. To this 100 ml of acetic acid is added.

5. Standard stock solution (1,1′,3,3′ Tetramethoxy propane (84mg/ml))

PROCEDURE:

To 0.5ml of plasma, 4ml of 0.083N sulphuric acid is added. To this mixture 0.5ml of 10% phosphotungstic acid is added and mixed, allowed to stand at room temperature for 5 minutes. The mixture is centrifuged at 3000 rpm for 10 minutes. The supernatant is discarded. To the remaining, 1 ml of TBA is added. The reaction mixture is heated at boiling water bath for 60 mts. After cooling, mixture is centrifuged at 3000 rpm for 15 mts. Supernatant is transferred to cuvette.

Standard MDA solutions are 2 µmol/L, 4 µmol/L, 6 µmol/L, 8µmol/L & 10 µmol/L and a blank were processed along with the test sample.

The absorbance at 530 nm was measured and subtracted from the blank. A calibration graph was prepared using MDA standard.

Reference Range:

Serum / Plasma MDA

0.03 to 3.88 µmol/L

GLYCOHEMOGLOBIN

METHODOLOGY:

Ion exchange resin method

PRINCIPLE

A haemolysed preparation of the whole blood is mixed continuously for 5 minutes with a weak binding cation-exchange resin. During this time, HbAo binds to the resin. After the mixing period, a filter is used to separate the supernatant containing the glycohaemoglobin from the resin.

Haemolysed Whole blood Preparation + Cation Exchange Resin $\xrightarrow{\text{Mix for 5 minutes}}$ Fast Fractions (HbA1a, HbA1b, HbA1c)

The Glycohaemglobin percent is determined by measuring the absorbance's at 415 nm of glycohaemoglobin fraction and the total hemoglobin fraction. The ratio of the two absorbances gives the percentage Glycohaemoglobin.

REAGENT COMPOSITION

REAGENT 1: GLYCOHAEMOGLOBIN ION EXCHANGE RESIN

Cation – Exchange Resin (pH 6.9)	8 m g / m l

REAGENT 2: GLYCOHAEMOGLOBIN LYSING REAGENT

Lysing Reagent	10 m M

REAGENT 3: GLYCOHAEMOGLOBIN CALIBRATOR

Calibrator	10%

REAGENT RECONSTITUTION

1. Glycohaemoglobin Ion Exchange Resin

The ion exchange resin is ready for use and prefilled in plastic tubes.

2. Glycohaemoglobin Lysing Reagent

The reagent is ready for use.

3. Glycohaemoglobin Calibrator Glycohemoglobin Calibrator is allowed to attain the room temperature.

The contents of each vial is dissolved in 1 ml of deionised water free of contaminants.

ASSAY PROCEDURE

STEP 1: HAEMOLYSATE PREPARATION

	CALIBRATOR	TEST
Lysing Reagent	500 µl	500 µl
Calibrator	100 µl	--
Sample / Whole Blood	--	100µl

STEP II: SEPARATION OF GLYCOHEMOGLOBIN

1. 0.1ml of the haemolysate is added from STEP 1 into the appropriately marked Ion-Exchange Resin tubes.
2. The filter separator is positioned approximately 2 cm above the liquid level in the tube.
3. The tubes are placed on the shaker and allowed to mix continuously for 5 minutes.
4. The tubes are removed from the shaker.
5. The filter separator is pushed until the resin is firmly packed.
6. The supernatant of each tube is poured into appropriately marked tubes.
7. Absorbance of each tube for **Glycohaemoglobin** at 415nm (450nm-420nm) against deionised water blank is read and recorded.

STEP III: TOTAL HAEMOGLOBIN FRACTION

The reading of analyzer is set to with deionised water.

	CALIBRATOR	TEST
Deionised water	5.0 ml	5.0 ml
Calibrator Haemolysate	20 µl	--
Sample Haemolysate	--	20µl

Mixed well, read and recorded the absorbance of calibrator, and sampled against a deionised water blank at 415 nm (405nm-420nm) for **Total Haemoglobin readings.**

CALCULATION

The ratio (R) of the Glycohaemoglobin absorbance to the total haemoglobin absorbance is calculated. The following equations are used to determine unknown concentrations.

$$Rc = \frac{\text{Absorbance of Calibrator (Glyco)}}{\text{Absorbance of Calibrator (Total)}}$$

$$Ru = \frac{\text{Absorbance of Unknown (Glyco)}}{\text{Absorbance of Unknown (Total)}}$$

$$\%\ \text{Glycohaemoglobin of unknown} = \frac{Ru}{Rc}\ \text{x value of Calibrator}$$

LINEARITY

The assay is linear upto 20% for glycohaemoglobin levels. For blood samples with total haemoglobin greater than 18g/dl sample should be diluted with deionised water before the assay.

NORMAL VALUES (Reference for guidelines)

	A1	**A1c**
NORMAL	6.0% - 8.3%	4.3% - 6.2%
GOOD DIABETIC CONTROL	7.5% - 9.0%	5.5% - 6.8%
FAIR CONTROL	9.0% - 10.0%	6.8% - 7.6%
POOR CONTROL	> 10%	> 7.6%

METHODOLOGY

DETERMINATION OF GLUCOSE IN SERUM

GLUCOSE OXIDASE / PEROXIDASE METHOD

PRINCIPLE

Glucose is oxidized to gluconic acid and hydrogen peroxide in the presence of glucose oxidase. Hydrogen peroxide further reacts with phenol and 4-aminoantipyrine by the catalytic action of peroxidase to form a red coloured quinoneimine dye complex. Intensity of the colour formed is directly proportional to the amount of glucose present in the sample.

$$\text{Glucose} + O_2 + H_2O \xrightarrow[\text{Oxidase}]{\text{Glucose}} \text{Gluconate} + H_2O$$

$$H_2O_2 + 4\ \text{Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{Peroxidase}} \text{Red Quinoneimine dye} + H_2O$$

Contents

L1 : Glucose reagents; 4x250 ml

L2 : Buffer reagent : 10ml

S : Glucose standard (100 mg/dl): 5ml

Reagent preparation:

2.5ml of Buffer reagent (L2) was added to 250ml of distilled water.

The contents of one bottle of glucose reagent (L1) was emptied into it, and mixed by gentle swirling and allowed to stand at room temperature for 30 minutes. This working reagent is stable for 60 days when stored at 2-8° C.

SAMPLE MATERIAL – Serum

PROCEDURE

Wave length / Filter: 505 nm to 546 nm Green

Temperature: 37° C/RT

Light path: 1 cm

The working reagent, distilled water, standard and sample were pipetted into clean dry test tube labeled as Blank (B), Standard (S), and Test (T) as follows:

Addition Sequence	**B (ml)**	**S (ml)**	**T (ml)**
Working Reagent	1.0	1.0	1.0
Distilled water	0.01	---	---
Glucose standard	---	0.01	---
Sample	---	---	0.01

Mixed well and Incubated at 37° C for 10 minutes. The absorbance of the standard (Abs. S) and Test sample (Abs. T) were measured against the blank, within 60 minutes at 505 nm.

Calculations:

Total glucose in mg/dl = $\frac{\text{Abs.T}}{\text{Abs.S}} \times 100$

Linearity:

This procedure is linear up to 500 mg/dl.

General system parameters

Reaction Type	:	Endpoint
Reaction Slope	:	Increasing
Wavelength	:	505nm
Incubation Temp	:	37°C/R.T
Sample Vol	:	10µL
Reagent Vol	:	1.0mL
Std. Concentration	:	100 mg/dL
Zero Setting With	:	Reagent Blank
Linearity	:	500 mg/dl

Reference value

Serum: Fasting 70-110 mg/dl

Post Prandial: <140 mg/dl

ESTIMATION OF CHOLESTEROL

ENZYMATIC METHOD PRINCIPLE

$$\text{Cholesterol Ester} + H_2O \xrightarrow{\text{Cholesterol Esterease}} \text{Cholesterol} + \text{Fatty Acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol Oxidase}} \text{Cholest-4-en-3-one} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + \text{4-Aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Red quinone} + 4\text{-}H_2O$$

The concentration of Cholesterol in the sample is directly proportional to the intensity of the red complex (Red Quinone) which is measure at 500 nm.

REAGENTS

Reagent 1 (Enzymes / Chromogen)

Cholesterol Esterease	≥ 200 U/L
Cholesterol Oxidase	≥ 250 U/L
Peroxidase	≥ 1000 U/L
4-Aminoantipyrine	0.5 mmol/L

Reagent 1A (Buffer):

Pipes buffer, pH 6.90	50 mmol/L
Phenol	24 mmol/L
Sodium Cholate	0.5 mmol/L

Standard (Cholesterol 200 mg/dL):

Cholesterol	2 g/L

STORAGE & STABILITY OF THE REAGENTS

When stored at 2° C - 8° C and protected from light, the reagents are stable until the expiry dates stated on the labels.

REAGENT RECONSTITUTION

The reagents are allowed to attain room temperature. The contents of one bottle of reagents 1 were dissolved with one bottle of reagent 1A and mixed by gentle swirling.

RECONSTITUTED REAGENT STORAGE & STABILITY

The reconstituted reagent is stable for 3 months when stored at 2° C - 8°C.

PROCEDURE

The samples and the reconstituted reagent were brought to room temperature prior to use.

The following general system parameters were used with this kit:

General system parameters

Reaction Type	:	Endpoint
Reaction Slope	:	Increasing
Wavelength	:	500 nm (492-550)
Flowcell Temp	:	30° C
Incubation	:	5 Min. at 37°C
Sample Vol	:	10 µL
Reagent Vol	:	1.0 mL
Std. Concentration	:	200 mg/dL
Zero Setting With	:	Reagent Blank

The instrument was set using above system parameters.

The reconstituted reagent, standard and the sample were dispensed in to test tubes as follows.

	Blank	**Standard**	**Test**
Reconstituted	1 mL	1 mL	1 mL
Standard	-	10 µL	-
Sample	-	-	10 µL

Incubated for 5 minutes at 37° C, mixed and read at 500 nm.

Linearity:

The method is linear up to 500 mg/dL

Reference value for Cholesterol

Serum / Plasma: Male = < 220 mg / dL

Female = < 200 mg / dL

ESTIMATION OF TRIGLYCERIDES

ENZYMATIC COLORIMETRIC METHOD

PRINCIPLE

$$\text{Triglycerides} + H_2O \xrightarrow[\text{Lipase}]{\text{Lipoprotein}} \text{Glycerol} + \text{Fatty Acid}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow[\text{Kinase}]{\text{Glycerol}} \text{Glycerol-3-Phosphate} + \text{ADP}$$

$$\text{Glycerol-3-Phosphate} + O_2 \xrightarrow{\text{GPO}} \text{Dehydroxyacetone Phosphate} + H_2O_2$$

$$2H_2O_2 + \text{4-Aminoantipyrine} + \text{ADPS} \xrightarrow{\text{Peroxidase}} \text{Red quinine} + 4H_2O$$

GPO – Glycerol – 3-phosphate oxidase

ADPS - N-Sulfopropyl-n-anisidine

The intensity of purple coloured complex formed during the reaction is directly proportional to the Triglycerides concentration in the sample and is measured at 546 nm.

REAGENTS

Reagent 1 (Enzymes / Chromogen)

Lipoportein Lipase	≥ 1100 U/L
Glycerol Kinase	≥ 800 U/L
Glycerol-3-Phosphate Oxidase	≥ 5000 U/L
Peroxidase	≥ 350 U/L
4-Aminoantipyrine	0.7 mmol/L
ATP	0.3 mmol/L

Reagent 1A (Buffer)

Pipes buffer, pH 7.50	50 mmol/L
ADPS	1 mmol/L
Magnesium salt	15 mmol/L

Standard (Triglycerides 200 mg/dL)

Glycerol (Trig.equivalent)	2 g//L

REAGENT RECONSTITUTION

The reagents are allowed to attain room temperature. The contents of one bottle of reagents 1 were dissolved with one bottle of reagent 1A, and mixed by gentles swirling and used after 5 minutes.

RECONSTITUTED REAGENT STORAGE & STABILITY

The reconstituted reagent is stable for 6 weeks when stored at 2°C-8°C.

PROCEDURE

The samples and the reconstituted reagent were brought to room temperature prior to use.

The following general system parameters were used with this kit:

General system parameters

Reaction Type	:	Endpoint
Reaction Slope	:	Increasing
Wavelength	:	546 nm (520-570)
Flowcell Temp	:	30° C
Incubation	:	5 Min. at 37°C
Sample Vol	:	10 µL
Reagent Vol	:	1.0mL
Std.Concentration	:	200 mg/dL
Zero Setting With	:	Reagent Blank

The instrument was set using above system parameters.

The reconstituted reagent, standard and sample were dispensed in to test tubes as follows:

	Blank	**Standard**	**Test**
Reconstituted reagent	1mL	1mL	1mL
Standard	-	10µL	-
Sample	-	-	10µL

Incubated for 5 minutes at 37°C. Mixed and read at 546nm. The final colour was stable for at least 30 minutes.

Linearity:

The method is linear up to 1000 mg/dl.

Reference value for Triglycerides

Serum/ Plasma Triglycerides 50-150 mg/dl

ESTIMATION OF HDL-CHOLESTEROL

PHOSPHOTUNGSTATE METHOD

PRINCIPLE

Chylomicrons, VLDL (Very Low Density Lipoproteins) and LDL fractions in serum or plasma are separated from HDL by precipitating with Phosphotungstic Acid and Magnesium Chloride. After centrifugation the cholesterol in the HDL fraction which,remains in the supernatant is is assigned with the enzymatic cholesterol method, using Cholesterol Esterase, Cholesterol Oxidase, Peroxidase and the chromogen 4-Aminoantipyrine/Phenol.

REAGENTS

Reagent 1(Enzymes/Chromogen)

Cholesterol esterase	≥ 200 U/L
Cholesterol Oxidase	≥ 250 U/L
Peroxidase	≥ 1000 U/L
4-Aminoantipyrine	≥ 0.5 mmol/L

Reagent 1A (Buffer):

Pipes buffer, pH 6.9	50 mmol/L
Phenol	24 mmol/L
Sodium Cholate	0.5 mmol/L

Reagent 2 (Precipating Reagent)

Phosphotungstic Acid	2.4 mmol/L
Magnesium Chloride	39 mmol/L

Standard (HDL Cholesterol 50mg/dL):

Cholesterol	0.5g/L

REAGENT RECONSTITUTION:

The reagents are allowed to attain the room temperature. The contents of one bottle of reagent 1 is dissolved into one bottle of reagent 1A, and mixed by gentle swirling till completely dissolved and used after 5 minutes.

RECONSTITUTED REAGENT STORAGE & STABILITY

The reconstituted reagent was stable for 3 months when stored at 2°C - 8°C.

PROCEDURE

The samples, the participating reagent 2 and the reconstituted reagent were brought to room temperature prior to use.

I. PRECIPITATION

The sample and precipitating reagent were dispensed into Centrifuge Tube as follows:

	Test
Sample	0.20 mL (200µL)
Precipitating Reagent 2	0.20 mL (200µL)

Mixed well and centrifuged at 3500-4000 rpm for 10 min. The clear supernatant was separated immediately and determined the Cholesterol content as for total cholesterol estimation.

II. CHOLESTEROL ASSAY

The following general system parameters were used with this kit:

General System Parameters

Reaction Type	:	End point
Reaction Slope	:	Increasing
Wavelength	:	500 nm (492-550 nm)
Flow cell Temp	:	30°C
Incubation	:	5Min 37°C
Sample Vol (Supernatant):		20 µL
Reagent Vol	:	1.0 mL
Std.Concentration	:	100 mg/dL (The Std. of 50 mg/dL is to be fed as 100 mg/dL to account for the dilution of sample in the precipitation step)
Zero Setting With	:	Reagent Blank

The instrument was set using above system parameters.

The reconstituted reagent, standard and supernatant were dispensed into test tubes as follows:

	Blank	Standard	Test
Reconstituted Reagent	1 mL	1 mL	1 mL
Standard	-	20 μL	-
Supernatant	-	-	20 μL

Incubated for 5 minutes at 37° C, mixed and read at 500 nm

Reference value in HDL – Cholesterol:

Serum/ Plasma; 40 – 60 mg / dL

ESTIMATION OF LDL CHOLESTEROL BY FRIEDEWALD EQUATION

[LDL CHOLESTEROL] = [Total Cholesterol]-[HDL Cholesterol]-[Triglyceride/5], all concentrations are in mg/dL.

VLDL = Triglyceride/5

Reference Value:

Serum/ Plasma LDL ; 100 – 129 mg / dL

VLDL < 40 mg / dL

Estimation of Urea : Diacetyl monoxime Method

Estimation of Creatinine : Jaffe's Method

Urine Sugar : Benedict's Method

Urine Albumin : Heat Coagulation And Sulpho Salicylate Method

RESULTS AND STATISTICAL ANALYSIS

The study shows that there is increase in serum MDA levels in all diabetes mellitus individuals.

From table 1 we infer that the mean value of MDA is high in diabetic patients (Mean 4.63 μmol/L) when compared to control group (Mean 3.61 μmol/L) and increase is statistically significant (P = 0.0001).

Table 2 shows significant increase in MDA levels of NIDDM group (Mean 4.8 μmol/L) as compared to IDDM group (Mean 4.46 μmol/L).

Table 3 shows significant elevation in MDA values along with increase in HbAIC values.

Table 4 shows significant elevation of Cholesterol, Triglyceride, LDL and VLDL in Diabetics when compared to control population. And significant decrease in serum levels of HDL when compared to control.

Table 5 shows positive correlation between MDA and Cholesterol, Triglyceride, LDL, VLDL in diabetics. Negative correlation between MDA and HDL in diabetics.

Table 6 shows significant increase in Cholesterol, Triglyceride, LDL, VLDL in poorly control diabetics and decrease in HDL cholesterol.

DISCUSSION

The mean value of plasma MDA is high in diabetic patients when compared to control group.

Increased lipid peroxidation in diabetes mellitus is due to excess formation of free radicals[40]. Hyperglycaemia in diabetics causes increased glycation of protein which itself act as a source of free radicals.

Metabolic derangements in diabetes lead to an increase in concentration of oxidizable substrates and compromised detoxification pathways.

The study shows that cases on insulin as therapeutic regime (IDDM) had lower mean MDA level (4.46 μmol/L) as compared to those on oral hypoglycaemics (NIDDM) (4.8 μmol/L) indicating lesser level of oxidative stress in diabetics on insulin.

Considering MDA levels among cases on the basis of their glycaemic status, significant correlation is seen between well controlled and poorly controlled diabetics (both in IDDM and NIDDM). MDA is

higher in individuals with poor glycaemic control compared to good glycaemic control.

For every 1% reduction in HbAIC, one can expect 35% reduction in microvascular complications[41].Which can be attributed to decrease in oxidative stress on treatment.

The metabolic parameters such as total Cholesterol, Triglycerides, LDL and VLDL values were more in diabetic groups than the control groups.

Mean value of serum HDL is decreased in diabetic group compared to control and decrease is statistically significant (P = 0.001).

Most common lipid disorder observed in DM is the presence of high plasma Triglyceride and low HDL cholesterol[42].

Insulin is the principal antilipolytic regulator, acting on hormone sensitive lipase.Without its action as in DM, lipolysis in adipose tissue is increased. As a result there is increased availability of NEFAS for reesterification in the liver to produce more triglycerides.

Lipoprotein lipase activity is less in insulin deficiency resulting in diminished Triglyceride clearance, impaired lipolysis of VLDL and reduced formation of HDL particles[43].

Insulin increases the number of LDL receptor. In insulin deficiency, the level of LDL receptors are low, which causes the increase in LDL cholesterol. LDL oxidation plays an important role in atherogenesis[44-49].

The uptake of LDL by macrophage(to form foam cell)is increased by

- Oxidation of LDL
- Derivitization of ApoB by glycosylation.
- Reaction with Malondialdehyde.

1) Oxidation of LDL

Oxidised LDL has many characteristics that potentially promote atherogenesis, in addition to the ability to be taken up rapidly by macrophages to form foam cells. It is a chemoattractant for circulating monocytes[50], both directly and also via stimulation of the release of monocyte chemoattractant protein-1 from endothelial cells[51].

The chemoattractant activity of LDL resides in its lipid moiety, and is attributable to lysophophatidylcholine generation during the conversion of LDL into its oxidized form. Oxidised LDL promotes the differentiation of monocytes into tissue macrophages by enhancing the release of macrophage colony stimulating factor from endothelial cells[52], and inhibits the motility of resident macrophages. It is a chemoattractant to T cells, although not for B cells, and consequently the atherosclerotic plaque contains primarily monocytes and T cells.

Unlike native LDL, oxidized LDL is immunogenic[53] and it is also cytotoxic to various cell types including endothelial cells, resulting in loss of endothelial integrity. It inhibits tumour necrosis factor expression, stimulates release of interleukin-1b from monocyte macrophages, and can inhibit endothelial cell dependent arterial relaxation. Oxidized LDL also activates matrix digesting enzymes, which plays a role in plaque instability.

2) Derivitization of ApoB by glycosylation.

The apopotein component of LDL, apoproteinB is subject to glycosylation[54]. Glycosylation occurs by reaction of glucose with free amino groups of amino acids such as the epsilon amino groups of lysine. These amino groups are critical for the normal recognition of

apoprotein B by the LDL receptor and on incubation of glycosylated LDL with cultured cells there was reduced uptake and degradation compared to control LDL due to reduced binding of glycosylated LDL to the LDL receptor. LDL glycosylation has been shown to affect LDL catabolism and LDL receptor binding, so prolonging the contact time of this highly atherogenic particle with vessel wall. Glycosylated LDL appers to be immunogenic,[55] the glycosylated LDL/ immune complex could damage the arterial endothelium.

3) Reaction with Malondialdehyde.

Cholesterol deposited in the atheromatous lesion is derived from plasma and LDL enters the arterial wall at a rate directly related to its plasma concentration[56]. The foam cells so characteristic of atheroma may have their origin in macrophage monocytes which have receptors for chemically altered LDL.

Unlike normal LDL recptors they are not down regulated by increasing cellular cholesterol concentration. One type of chemically altered LDL taken up by macrophages[57] resulting in massive cholesterol accumulation in those cells is malondialdehyde-modified LDL[58].

An attractive hypothesis would be that malondialdehyde, a stable end product of the prostaglandin cascade, released from platelets or produced by lipid peroxidaion at sites of injury of the arterial wall could lead to chemical modification of LDL rendering it recognizable by macrophage receptors leading to cholesterol accumulation in those cells. Lipid laden macrophages form the foam cell that contribute to development of fatty streaks and the atherosclerosis[59]. Oxidative stress occurs at an early stage in the disease pathway. It predates the complications .

The oxidative stress in IDDM and NIDDM is evidenced by increased levels of plasma MDA, so intensive glycaemic control is well established as a standard of care for patients with diabetes achieving and sustaining glucose control can substantially reduce the risk of microvascular complications in diabetes mellitus[60]. Oxidative stress in terms of MDA is increased in NIDDM when compared to that in IDDM. So insulin therapy has a beneficial effect on oxidative stress.

CONCLUSION

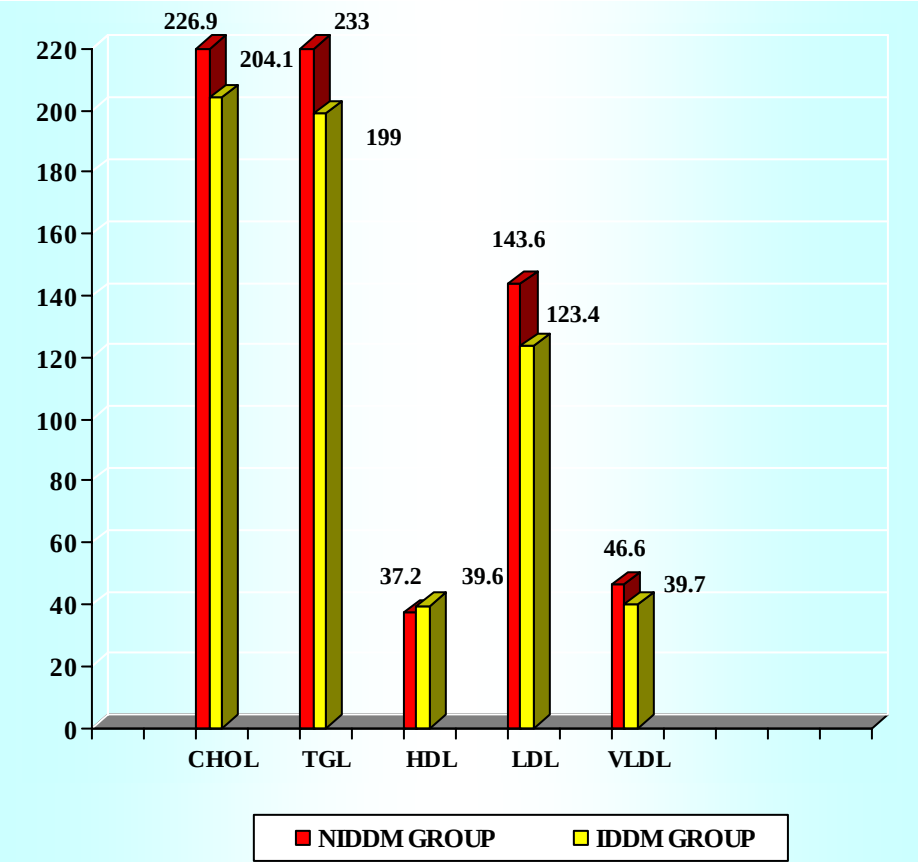
It is evidenced that the level of MDA is increased in both types of DM.There is significant increase in levels of plasma Total cholesterol, TGL,LDL and VLDL and significant decrease in levels of HDL in both types of DM.

To conclude in the era of modern medicine diabetic complications demand prevention and management.

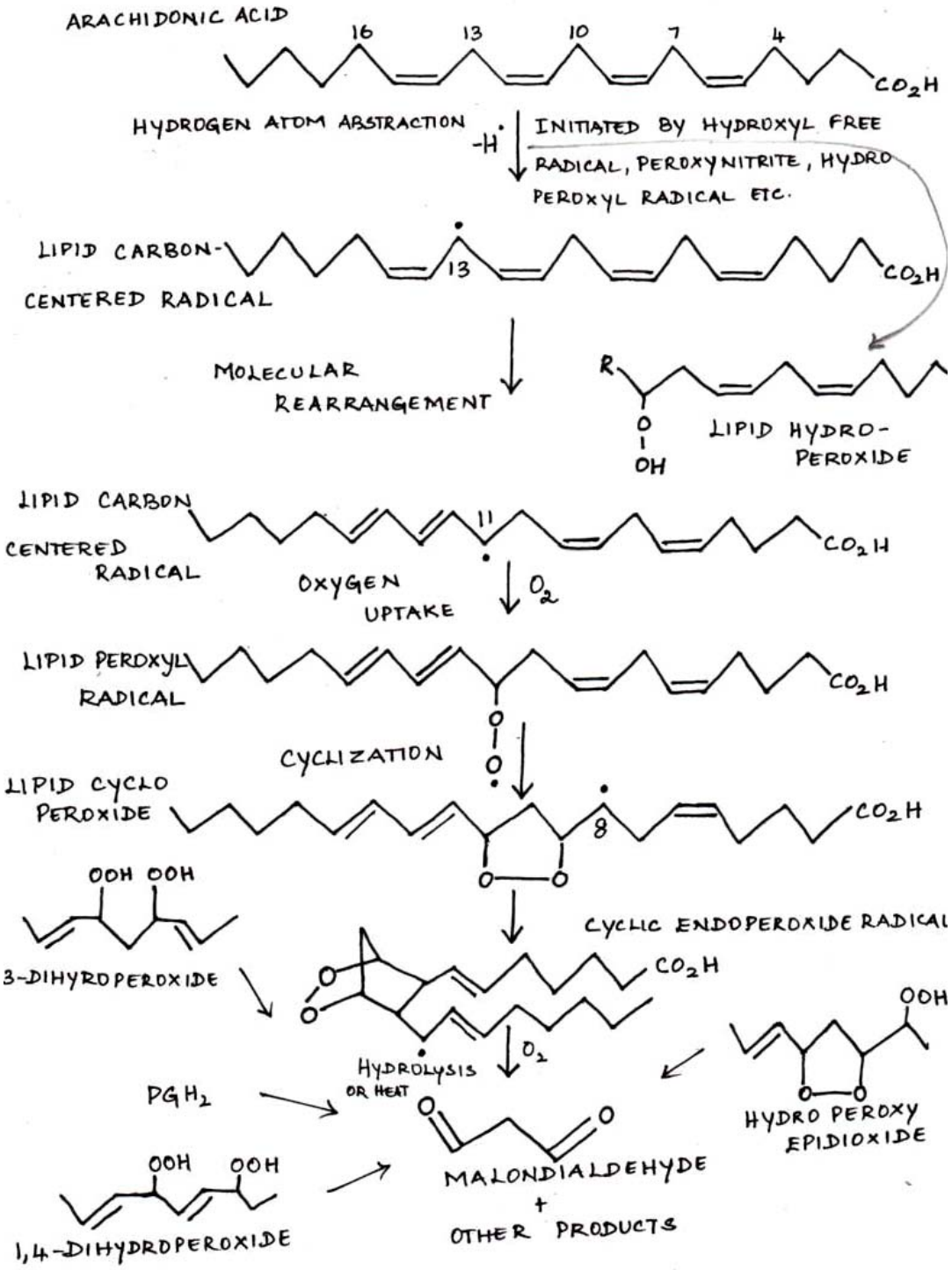
The estimation of lipid peroxide along with lipid profile in diabetes mellitus is very useful as it may serve as a useful monitor to judge the prognosis of the patient. The detection of risk factor in the earlystage of the disease will help the patient to improve and reduce the morbidity rate.

It is with this background that the ray of hope provided by the considerable evidence suggesting the role of prevention of increased lipid peroxidation could offer feasible and cost effective way to reduce the prevalence of diabetic complications

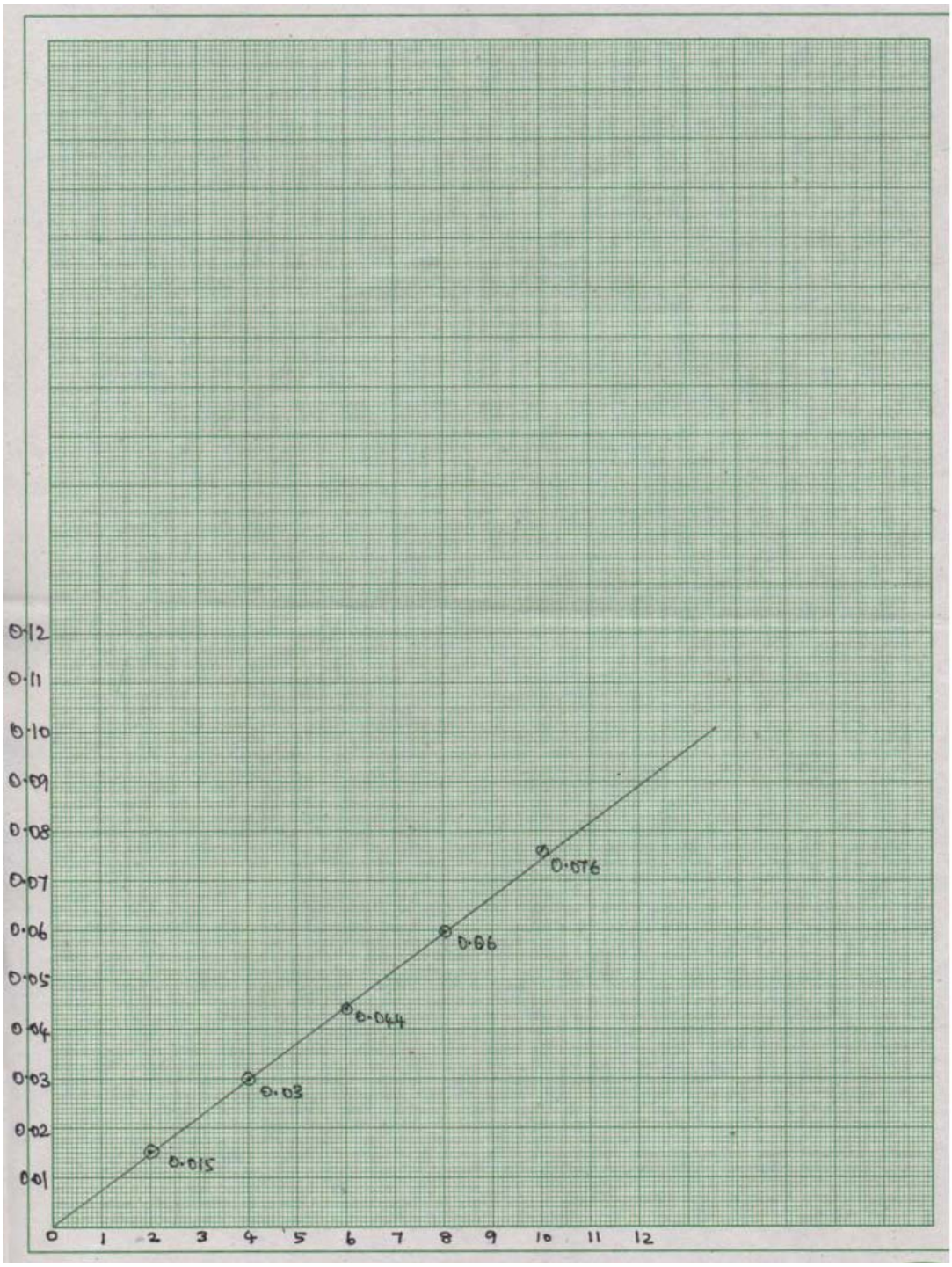
LIPID PROFILE IN NIDDM & IDDM CASES



LIPID PEROXIDATION OF PUFA TO MDA



STANDARD CALIBRATION GRAPH FOR MDA



Xaxis : 1cm = 1µmol/litre
Y axis: 1 cm = 0.01 od

CONTROL

									B/Sugar mg/dl			CHOL	TGL	HDL	LDL	VLDL	Urea	creat	URINE	
S.NO	NAME	AGE	SEX	BP mmHg	HT cm	WT kg	BMI	MDA µ mol/L	F	PP	HbA_1C %	mg/dl							AB	Sugar
1	Jothi	42	F	110/70	150	52	23.1	3.5	64	132	4.7	172	105	46	105	21	28	0.8	NIL	NIL
2	Kannagi	41	F	110/70	148	53	24.2	3.5	81	116	4.6	161	88	42	101	18	32	0.9	NIL	NIL
3	Mariyapushpam	46	F	110/70	142	38	18.9	3.6	66	110	4.8	154	92	42	104	18	20	0.6	NIL	NIL
4	Kalaichelvi	49	F	120/80	146	50	23.4	3.7	90	130	5.4	184	156	46	107	31	28	0.8	NIL	NIL
5	Rajeswari	40	F	120/76	149	48	21.6	3.4	72	112	4	147	94	42	86	19	18	0.7	NIL	NIL
6	Arulayi	50	F	120/70	152	58	25	3.7	93	105	5.5	186	142	42	116	28	28	0.7	NIL	NIL
7	Thilagavathy	36	F	120/70	153	54	23	3.7	75	100	5.6	163	99	48	95	20	30	0.8	NIL	NIL
8	Mariyammal	57	F	120/80	152	56	24.2	3.6	76	104	5	156	132	42	88	26.4	22	0.7	NIL	NIL
9	Minnalkodi	50	F	120/70	148	54	24.6	3.5	80	122	4.9	158	134	42	89	26.8	26	0.8	NIL	NIL
10	Saroja	60	F	120/70	152	53	22.9	3.6	66	106	4.8	136	106	41	74	21	28	0.7	NIL	NIL
11	Ratriyabeevi	48	F	120/80	158	60	24.1	3.6	74	112	4.8	144	110	42	80	22	26	0.8	NIL	NIL
12	Vidya	33	F	120/70	148	50	22.83	3.6	106	120	4.7	126	99	46	60	20	24	0.6	NIL	NIL
13	Latha	55	F	120/80	160	61	23.8	3.6	68	101	4.8	146	86	42	87	17	26	0.7	NIL	NIL
14	Umamaheswari	24	F	110/70	156	48	19.7	2.9	76	116	5	138	98	43	65	20	28	0.6	NIL	NIL
15	Leemarose	42	F	120/70	153	49	20.9	3.7	74	110	5.1	142	92	42	81	19.4	29	0.8	NIL	NIL
16	Pankajam	53	F	130/80	148	50	22.8	3.6	80	98	4.6	156	102	42	94	20.4	28	0.7	NIL	NIL
17	Kavitha	27	F	110/70	156	52	21.4	3.4	76	102	4.6	138	96	44	75	19.2	24	0.6	NIL	NIL
18	Saroja	70	F	120/80	154	55	23.2	3.6	98	116	4.9	182	136	41	114	27	32	0.9	NIL	NIL

									B/Sugar mg/dl			CHOL	TGL	HDL	LDL	VLDL	Urea	creat	URINE	
S.NO	NAME	AGE	SEX	BP mmHg	HT cm	WT kg	BMI	MDA µ mol/L	F	PP	HbA_1C %	mg/dl							AB	Sugar
19	Malliga	22	F	110/70	148	52	23.7	3.4	76	108	4.8	124	89	46	60	18	24	0.6	NIL	NIL
20	Gunasundari	40	F	110/74	153	54	23.1	3.3	76	118	4.6	136	98	44	74	20	28	0.7	NIL	NIL
21	Susila	53	F	120/80	138	46	24.2	3.7	82	122	6	144	106	42	81	21.2	28	0.7	NIL	NIL
22	Gunavathy	30	F	110/70	159	60	23.8	3.4	62	84	4.3	132	98	44	68	20	24	0.7	NIL	NIL
23	Sumathy	41	F	110/70	162	60	22.9	3.6	74	106	4.9	154	102	43	91	20.4	24	0.7	NIL	NIL
24	Meenatchi	38	F	120/70	156	57	23.4	3	72	112	4.8	130	96	42	69	19.2	26	0.6	NIL	NIL
25	Rajathi	48	F	120/76	154	56	23.6	3.7	80	116	5.2	144	104	45	76	23	28	0.7	NIL	NIL
26	Rengasamy	65	M	130/80	162	64	24.4	3.7	86	125	5.3	170	128	40	63	25.6	29	0.9	NIL	NIL
27	Rajendran	51	M	120/80	153	60	25.6	3.6	87	98	4.9	141	117	42	76	23	28	0.8	NIL	NIL
28	Shankaran	42	M	130/80	165	68	25	3.6	78	108	4.8	144	109	44	78	21.8	28	0.8	NIL	NIL
29	Sambatham	65	M	110/70	150	56	24.8	3.5	62	105	4.6	148	86	40	91	17	26	0.8	NIL	NIL
30	Padmanaban	37	M	116/76	153	55	23.5	3.6	72	108	4.9	150	98	46	85	19.6	27	0.7	NIL	NIL
31	Sundaram	50	M	130/80	154	62	26.1	3.7	90	116	5.4	160	110	42	96	22	28	0.9	NIL	NIL
32	Swaminathan	64	M	130/80	160	64	25	3.6	78	118	4.7	170	132	41	113	26.4	30	0.9	NIL	NIL
33	Ramu	38	M	120/70	148	54	24.6	3.6	76	104	4.6	146	134	43	77	26.8	22	0.7	NIL	NIL
34	Murali	44	M	120/70	160	62	24.2	3.6	90	108	5.3	150	96	43	88	19	28	0.8	NIL	NIL
35	Logeshwaran	33	M	120/70	158	52	20.8	3.5	76	98	4.7	136	98	45	71	20	24	0.6	NIL	NIL
36	Vijay Anand	32	M	120/70	148	50	22.8	3.5	74	112	4.7	154	102	44	90	20.4	26	0.7	NIL	NIL
37	Deva	21	M	110/70	156	54	22.2	3.4	68	106	4.4	132	84	45	70	17	24	0.6	NIL	NIL

									B/Sugar mg/dl			CHOL	TGL	HDL	LDL	VLDL	Urea	creat	URINE	
S.NO	NAME	AGE	SEX	BP mmHg	HT cm	WT kg	BMI	MDA µ mol/L	F	PP	HbA_1C %	mg/dl							AB	Sugar
38	Valluvan	36	M	120/70	158	57	22.8	3.6	78	120	4.6	136	88	44	74	18	28	0.7	NIL	NIL
39	Karthikeyan	27	M	110/70	154	56	23.6	3.6	77	118	4.6	146	120	46	76	24	22	0.7	NIL	NIL
40	Johnbritto	25	M	110/70	156	58	23.8	3.7	80	113	5	144	88	43	83	18	23	0.6	NIL	NIL
41	Ayyadurai	63	M	130/80	158	60	24.1	3.8	82	124	5.1	155	107	44	90	21	30	0.9	NIL	NIL
42	Ramasamy	50	M	120/70	156	60	24.6	3.4	68	108	4.4	160	124	46	90	24.8	30	0.8	NIL	NIL
43	Muthusamy	70	M	130/80	158	56	22.4	3.6	90	124	5.3	172	126	42	105	25.2	36	0.9	NIL	NIL
44	Velu	56	M	120/80	154	51	21.5	3.6	86	114	5.1	147	108	43	83	21.6	26	0.8	NIL	NIL
45	Chandran	35	M	120/70	164	63	23.5	3.6	88	128	4.9	138	112	44	72	22.2	24	0.7	NIL	NIL
46	Jaganathan	55	M	120/80	162	60	22.9	3.8	78	126	4.8	154	128	42	86	26	28	0.8	NIL	NIL
47	Gajendran	24	M	116/70	160	56	21.8	3.2	72	112	4.7	130	98	45	65	19.6	26	0.6	NIL	NIL
48	Ramamoorthy	53	M	120/80	156	58	23.8	3.3	68	110	4.4	152	106	42	89	21	24	0.7	NIL	NIL
49	Rengaraj	60	M	120/80	149	53	23.8	3.5	78	114	4.6	154	96	43	92	19	34	0.8	NIL	NIL
50	Elavarasan	47	M	120/80	156	59	24.2	3.4	118	118	4.4	147	98	45	82	19.6	24	0.8	NIL	NIL

BIBLIOGRAPHY

1. Michael L. Bishop, Edward P. Fody, Larry E. Schoeff. Clinical Chemistry Technique, Principle correlations 6th edition 2009, Woltees Kluwee (India) New Delhi :13;314.

2. Altamer E, Vandemisle G and Chicca D. Increased lipid peroxidation in type II poorly controlled diabetic patients, Diabete: Etab 18(4); 264-671.

3. K Park MBBS, MS, Park's text book of preventive and social medicine 20th edition 6; 342-343.

4. Carl A. Burtis, Edward R. Ashwood, David E. Bruns. Tietz text book of clinical chemistry and molecular diagnostics 25; 854.

5. Shauna C. Anderson PhD, Susan Cockayne Phd clinical chemistry concept and applications 2003, 10; 159.

6. P.K. Mohanty, Bhavana Bai. Effect of short term insulin regimen on lipid peroxidation in type 2 diabetes mellitus, National Journal of Basic Medical Sciences, vol 1, Issue I; 15-20.

7. Lester Packer, Peter Rosen, Hans J. Trits Chler, George L. King, Angelo A 2221 Antioxidants in diabetes management UNESCO MCBN 15A 2000; 3:42-45.

8. Agarwal S, Baneejee S and Chattejee S.N. Effects of oxygen and ferrous sulphate induced lipid peroxidation in liposomae membrane. Ind J Biochem and Biophysics 21, 331-334.

9. Chattergee S.N. Agarwal S and Amitkuma, Membran lipid peroxidation and its pathological consequence. Ind J. of Biochem and Biophysics 25, 31.

10. Steiner G. Arthroscleroses, the major complication of diabetes. In urnaic M, Hollenberg CH, Steriner G, eds. Comparison of type 1 and type 11 diabetes: similarities and dissimilates in etiology, Pathogenesis and complications, New York: Plenum press: 1985; 277-97.

11. William T. Marshall, Stephen K Bangert Clinical Biochemistry, Metabolic and Clinical aspects, second edition. Churchill Livingstone, 2008: 946-947.

12. Nerup J. The clinical and immunological association of diabetes mellitus and Addison's disease. In: Bastenine PA, Gepts W, Addison AM, eds, Immunity and Auto immunity in Diabetes mellitus Amsterdam excerpta medica 1947: 149-183.

13. Gepts W Pathologic anatomy of the pancreas in Juvenile diabetes mellitus, Diabetes 1965; 14: 614-633.

14. Nicholces A Boon, Nicki R College, Brian R Walka, John AA Hunter Davidson's Principles and Practice of Medicine. 20th edition; 21: 808.

15. Ardhendu Sinha Ray, Abhisekh SinhaRay, Essential of internal medicine first edition. IX; 325-326.

16. Vivian A, Fonseca MD, Clinical Diabetes, Translating Research into practice, Type 1 diabetes: immunology and Genetics: 2006,2:6.

17. Donald Voet Judith G. Voet, Charlotte W Pratt, Fundamental of Biochemistry, 2nd Edition, John Wiley & sons pte ltd, 2006; 21:779.

18. Allen R. Myers, M.D, NMS medicine 5th edition BI Publications 2005; IV A: 515.

19. Praveen Kumar, Michael Clark, clinical medicine, A text book for medical students and doctors 3rd edition. 17; 834.

20. V. Mohan, Gundu HR Rao, Type 2 diabetes in South Asians: Epidemiology Risk factors and prevention 8th Edition 2007. Jaypee Brothers Medicinal publishers (P) ltd, 27: 369-370.

21. V. Seshiah, VS Ganesan, CV Hainarayan, V. Balaji, MadhuriBalaji, A hand book of diabetes mellitus. All Indian publishers 2nd edition, 2005; 2: 11.

22. John W Baynes PhD, Marker K Dominiczak MD, Medical Biochemistry 3rd edition Mosbx Elsevier, 2009; 21: 269-270.

23. Lee Gold Man, Dennis Ausiello Cecil text book of medicine 22nd edition vol II; 242: 1429.

24. Jain AP and Gupta DP, Study of blood lipids in diabetes without any manifest vascular complications. J.Dia Asso Ind Vol XX, 29-34.

25. Y Sachdev, Clinical Endocrinology and DM,Ist edition , Volume I ; 2008: 41, 394

26. Stewart ML, LakerMF, Dyer RG, Game FL, Mitcheson J, Winocour PH Alberti Kamm, Lipoprotein Compositional abnormalities and insulin resistance in type 2 diabetic patients with mild hyperlipidaemia Arteriosclero; B: 1046-52.

27. DM Vasudevan, Sreekumaris. Text book of Biochemistry for Medical students 5th edition, 2007; 13: 161.

28. John Betteridge, Lipids and Vascular disease current issues marten Dunitz ltd, UK, 2000, 4: 39-41.

29. Aruoma OI, Halliwell B, Butter J, Honey BM, Apparent inactivation of α_1, antioproteinase by sulphur containing radicals derived from penicillamine. Biochem pharmacol 1989; 38: 4353-4357.

30. Lesster Packer, Peter Rosen, HansJ, Tritschler, George L King,Angelo A 221, Antioxidants in diabetes management oxidative stress Application of Diabetes and to evaluation of the effects of anti oxidants. UNESCO-California – 2000; 3: 42-87.

31. Sinclari AJ, Lunec J, Free radicals, Oxidative stress and diabetes mellitus. In: Winyard P, Blake DRek Immunopharmacolony of Free radicals species, London: Academic press 1995: 183-198.

32. Hanachi et al, Oxidative stress and diabetes mellitus.2004,Indian Journal of basic medical sciences ,volume I;15-17

33. Gawel S , Wardas M ,Nied work E ,Wardas P ,Malondialdehyde as a Lipid Peroxidation marker .wiadtek,2004; 57 (9-10) :453-5

34. Del Rio D, Steward AJ ,Pellegrni N,A review of recent studies on Malondialdehyde as toxic molecule and biological marker of Oxidative stress

35. Janero DR ,MDA and TBA reactivity as diagnostic indices of lipid peroxidation and Peroxidative tissue injury .Free radic Biol Med.1990;9:515-40

36. Ayse Nus Torun ,Sevsen Kulak Sizoglw ,Mustafa ,Kulak SizoGlw,Barisonder Pamuk ,Elif Isbilen , Neslihan Bascil Tatuncu ,Serum total antioxidant status and lipid Peroxidation marker MDA levels in overt and subclinical hypothyroidisam , August 2008 ;10:1365-2265

37. Steinberg D,Parthasarathy S , Carew TE , et al .Beyond Cholesterol modification of low density lipo protein that increase its athrogenicity .N .Eng J Med 1989; 320:915-924

38. Heinecke JW ,Mechanisms of Oxidative damage of LDL in atherosclerosis curropin lipidol 1997;8:268-274.

39. Esterbauer H. Gebicki J,Putil H, Jurgens G. The role of lipid peroxidation and anti oxidants in oxidative modification of LDL. Free rad Biol. Med 992:13:341-390.

40. N.P.Suryawanshi, A.K.Bhutey, A.N.Nagdeote,A.A.Jadhav and G.S.Manoorkar. Study of lipid peroxide and lipid profile in Diabetes Mellitus. Indian journal of clinical Biochemistry, 2006;21:126-130.

41. Sayday SH, Ebarhardt, MS, Loria cm, et al, Age and burden of death attributable to diabetes in the united states. AM. Epideiol: 2002; 156:714-719.

42. Anne Peters Harmel, MD, Ruchi Mathur MD, Davinson's diabetes mellitus diagnosis and treatment, 5th Edition 2004; 8: 246.

43. Cathcard MK,Folcik VA, Lipoxygenases and atherosclerosis.. Protection versus pathogenesis, Free Radic Biol Med 2000;28:1726-34.

44. Kuhn H, Chan L, The role of 15-Lipoxygenase in atherogenesis; pro and antiaatherogenic actions, Curropin Lipidol 1997;8:111-17.

45. Yia-Herttuala S, Palinski W, Rosenfeld ME, et al. Evidence for the presence of oxidatively modified low density lipoprotein in atherosclerotic lesions of rabbit and man.J.clinInvest 1989;84:1086-95.

46. Parthasarathy S,RankinSM, Role of oxidized LDL in atherogenesis Prog Lipid Res 1992:31:127-43.

47. Berliner TA, Navab M, Fogelman AM et al. Basic Mechanisms oxidation, Inflammation and genetics, Correlation 1995;91:2488-96.

48. BerlinerTA, HeineckeJW, The role of oxidized LP in atherogenesis. Free Rad Biol Med 1996;20:707-27.

49. Steinberg D. Oxidative Modification of LDL and atherogenesis, Circulation 1997;95:1062-71.

50. Quinn M.T,Parthasarathy S,Fong L.G and Steinberg D.Proc. Natl.Acad.Sci. USA; 84:2995-2998

51. Cushing S.D.,Berliner J.A.,Valente, A.J et al(1990) Proc. Natl.Acad. Sc.USA;87:5134-5138

52. Rajavashisth T.B, Andalibi,A, Territo,M.c;Berliner,J.A,Navab,M Fogelman,A.M and Lusis, A.J (1990) Nature (London) 344, 254-257.

53. Palinski W, Rosenfeld, M.E., Yla- Herthula S et al(1989) Proceedings of the National Academy of sciences USA 86:1372 - 1376

54. Gonen B,Baenziger J,Schonfeld G, Jacobson D, Farrar P 1981 Non-enzymatic glycosylation of low density lipoprotein in vivo: effects on cell-interactive properties. Diabetes 30:875-878.

55. Witztum J L, Steinbrecher UP, Fisher M, Kesaniemi A 1983 Non enzymatic glycosylation of homologous low density lipoprotein and albumin renders them immunogenic in the guinea pig. Proceedings of the National Academy of sciences USA 80:2757-2761.

56. Nicoll A, Duffield R G M , Lewis B 1981 Flux of lipoproteins into human arterial intima. Atherosclerosis 39:239-242.

57. Goldstein J L, Ho Y K,Basu S K,Brown MS, 1979.A binding site on macrophages that mediates the uptake and degradation of acetylated low density lipoprotein producing massive cholesterol deposition. Proceedings of the National Academy of Sciences USA 76:333-337

58. Fogelman A M, Shechter I, Seager J, Hokom M, Child J S, Edwards P A 1980 Malondialdehyde alterations of low density lipoprotein leads to cholesteryl ester accumulation in human monocyte-macrophages. Proceedings of the National Academy of Sciences USA 77: 2214 – 2218.

59. Lowrence A.Kapalan,AmadeoJ Pesce, StevenC.Kazmierczak, Clinical Chemistry,Theory, Analysis, Correlation 4th Edition2003;33:630-31.

60. Stratton IM, Alder A I, Neil H A W, Matthews DR Monley SE, Cull CA, Hadden D, Turner RC, on behalf of UKPDS group, Assocation of Glycaemia with macrovascular and microvascular complications of type 2 diabetes. BMJ 2000; 321:405-412.